

The Shackleton incident could profit international law

THE incident at sea last week, in which the British research ship *Shackleton* had to make rapidly for port in the Falkland Islands after a warning shot had been fired across its bows by the Argentine destroyer *Almirante Storni*, must not be seen simply as an isolated event connected with a territorial dispute and a trigger-happy captain. In recent years the freedom of marine scientists to do pretty well what they wanted even within sight of foreign territory has been rapidly eroded, and there is no sign that when the Law of the Sea conferences have run their course life will be much easier for those who need to come fairly close to foreign coastlines. The point which should be abundantly clear is that Argentina is prepared (not for the first time) to take strong action against infringements of what she regards as her own economic zone by research vessels. She, along with many other countries, has sought to have the power to regulate scientific research within that zone.

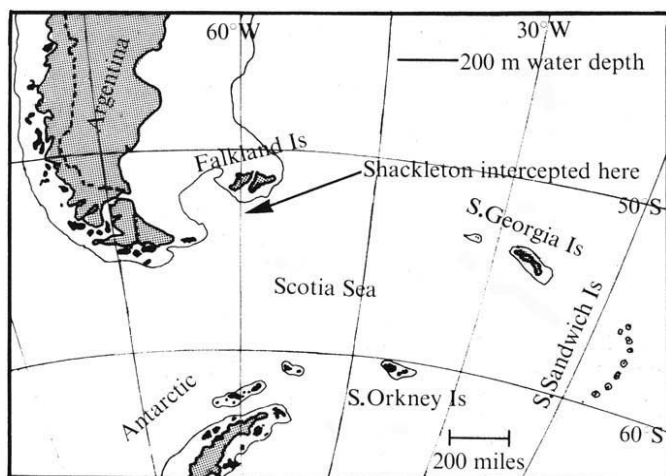
The research conducted from *Shackleton* used conventional geophysical techniques: magnetometry, gravimetry and refraction and reflection seismology. Most of it was being carried out in deep water in the Scotia Sea, and the programme, under the supervision of Birmingham University, has been running for many years. The purpose of the work is to attempt to put the unique area off South America into a plate tectonic context.

Those countries, Britain included, which have attempted

to produce guidelines for international discussion on what is pure and what is applied research would almost certainly rate *Shackleton*'s operations as 'pure'. But it is not as simple as that. Theories of the regional evolution of the Earth's crust have a lot in them for the economic geologist and thus for the prospecting industry; a clear picture of past geological history will not point a finger at exactly where hydrocarbons will be found, but it will provide some helpful signposts. Reasonably, scientists may protest that their work is the pursuit of knowledge for its own sake, that it is not supported by commercial interests, and that their results will be available internationally. But equally reasonably, countries with inadequately developed technology and expertise to capitalise on this information may feel themselves consistently outmanoeuvred when it comes to negotiating exploration rights. Their only defence then may be to demand some sort of technical assistance in exchange for access.

In a month's time the Law of the Sea Conference will resume in New York, and among other things will attempt to come to terms with a 'single negotiating text' including draft articles on marine scientific research. It is likely that the coastal state in whose economic zone another nation wishes to perform research will be called upon to decide whether the research is 'pure' or 'applied' and, in the former case, presumably to grant permission without too many strings attached. An appeal procedure to international experts is proposed if agreement cannot be reached. This seems a much better approach than any attempt by the United Nations to produce a universally acceptable list of topics to be regarded as pure.

It will be a pity if the *Shackleton* affair cannot be viewed at the conference as more than a territorial dispute. For, squabbles apart, it provides a rather good concrete example of the sort of research operation that is going to give rise to international disagreement; yet there is no reason why intelligent give and take on technology transfer and technical advice should not resolve such disputes. If this incident could be freed from its accretions of diplomatic conflict and considered simply as an ideal case for the Law of the Sea Conference to consider when legislating, some good could come of it. But it will need a certain detachment from the British delegation. □





Looking at the job first

In Nature last November, Cyril Cooper of the Institution of Professional Civil Servants highlighted the discontent among UK Civil Service scientists, and argued that it stemmed largely from differences they experience in salary and career progression compared with graduate administrators. David Budworth of the Confederation of British Industry replies.

THE NEWS that the Expenditure Committee is to examine the progress made in implementing the 1968 Fulton Report on the Civil Service is indeed welcome. Although the stimulus has no doubt been the rising public indignation at the advantages in terms of pay, security, and perhaps most conspicuously pensions that civil servants enjoy, the terms of reference of the investigation seem likely to be sufficiently wide to permit the Committee to tackle the problem of civil service scientists, which has been consistently ducked in recent years.

While it may well be good trade union tactics to argue, as Cyril Cooper does (November 20, 1975, page 186) that the scientific civil service is really just an ordinary part of the higher reaches of the service and should be treated as such, this approach flies in the face of economic reality and common sense. It is certainly also in flat contradiction of the Fulton principle of "look at the job first". Regrettably perhaps, but undeniably, the number of jobs available for administrators in the civil service has increased, and with it the security and career prospects of the incumbents, in our developing welfare state in which authority is looked upon to solve all the problems.

Science having conspicuously failed in its overblown claims to solve these problems, the opportunities for scientists have not expanded in a similar way. As David Davies has reminded us (May 22, 1975, pages 293-296), the scientific civil service has been static in numbers for the best part of twenty-five years, and it suffers from considerable internal embarrassments caused by its over-concentration of older scientists. Yet these under-used and probably unhappy people are a considerable national resource of talent and experience. Diverting them to supposedly useful tasks in their present locations is unlikely to do much of value. They must be moved out to

contribute to solving our national problems in the places where it is increasingly being recognised that something effective can be done: industry and the schools.

A Select Committee such as the Expenditure Committee is perhaps the only type of body which can look at these problems relatively free from civil service establishment influence. The Fulton Committee itself brushed aside the suggestions made to it, significantly including some from inside the service itself, that the scientific civil service would benefit from not being a career service; and Lord Rothschild's forthright comments on the immobility of scientific civil servants were safely defused by the terms of reference given to the Bondi task force which was set up to tackle the problem.

It is greatly to the credit of that body and of its chairman, whose personal commitment to mobility has been amply proved by deeds as well as words, that it did manage to rise slightly above its terms of reference and record its view that permanent moves were, in the end, more important than the immensely difficult and therefore marginally effective secondments to which it was nominally confined. Ironically perhaps, the work so far of the interchange unit set up in the Civil Service Department to keep the flame of scientific mobility alive, seems to have proved the critics of civil service privileges right, for the movement into the service has greatly exceeded the movement out.

Of course, as is apparent from Cyril Cooper's article, all pretence that the "fair comparison with outside employment" system for determining pay applies to civil service scientists has been abandoned, following the report of the late Pay Board's Advisory Report No. 3 of April 1974. This body again tamely accepted that something closely approximating to parity should exist between specific grades in the

administration and scientific groups of the service.

The reforms in the scientific civil service which are needed to effect a long-term cure are fairly clear in outline, and have been put forward so often that there is no need to repeat them here; but what of the short and medium term? We cannot afford to wait another 30 years or so, even assuming that the necessary changes are made, for the situation to right itself. Something must be done before that, and preferably soon. The former Department of Trade and Industry admitted in a burst of frankness in 1972 that it wanted to reduce the burden that its laboratories placed on public expenditure, but its attempts to do so by selling contract research cannot have made much impact. The unproductive PSOs mentioned by David Davies can hardly be declared redundant, particularly with unemployment at its present levels; but could not some way be found of getting them out to somewhere where their undoubted abilities might be put to use? From all accounts, good quality scientists and mathematicians are still not coming forward for teaching posts, and even when they do it seems that local authorities cannot afford to pay them. Is it totally beyond the wit of what is supposed to be the best administrative civil service in the world to devise some methods of utilising those on the public payroll to do the jobs which need to be done?

Again, if the Government is serious in its commitment to putting the needs of manufacturing industry above almost all other calls on its resources, there will be a need to move more brain power, and to move it pretty quickly, to where it can be commercially effective. Some scientists in government laboratories have proved themselves to be effective in marketing their services. Perhaps they could be equally useful in marketing the mechanical engineering and other products by which we live. Or will the real gap in our country, that between those who live on taxpayers' money by the skilled deployment of political argument, and those who live by exploiting their entrepreneurial abilities in some kind of market, be allowed to get even worse, so that we all eventually starve?

"Genetics" and how we made it

Steven Rose, Professor of Biology at the Open University, looks at the origins and purpose of the OU's new genetics course

SINCE the beginning of this month some 900 Open University students have been settling down for their standard 12 hours study a fortnight to follow a new second level Genetics Course—a set of 16 sequential study Units ranging in context from classical genetics by way of molecular and cytological genetics to population and human genetics, each with an accompanying TV and radio programme and a series of "home experiments". What makes the Genetics Course different from all the rest of the OU science teaching, however, is that it is the result of almost 3 years of collaboration between the Biology Department of the OU and the Department of Genetics at Birmingham, the Unit of Genetics at Hull, the School of Biology at Sussex and a member of the biology staff at the University of York. Teaching staff from the four collaborating universities were released from most of their other teaching duties in order to work on the production of this Course—a participation made possible by a grant from the Nuffield Foundation, which has an interest both in promoting inter-university teaching projects and in the development of the type of structured teaching that the OU has pioneered at University level. But the involvement was not just that of individuals, for each participant had the support of his department and access to its own particular area of genetic and teaching expertise. The result has been a novel Course that can be used both by the OU and in many other teaching institutions.

There were several reasons for choosing genetics for this experiment. The modern synthesis of genetics underlies most areas of biology. It is a subject that, throughout its history, and to the present day, has been interwoven with social and philosophical controversy concerning its interpretation and implications. It is very well suited for a problem-oriented, student-active teaching approach. And finally, a prosaic but important point is that not even all universities, and certainly rather few polytechnics and colleges, have biology schools large enough to be able to teach Genetics across the full range, from the molecular to the population and human levels. Hence the brief of the Joint Universities Genetics Course Team has been to prepare a Course that will eventually be able to be used in whole or in part by a very wide range of institutions even when they do not have a full range of genetic

skills represented amongst their teaching staff.

Working as an inter-university team has had both its pleasures and its perils. We came into the venture with differing views, based on our differing experiences, not merely on *what* should be taught but on *how* it should be taught. University teachers tend to be individualists, but, meeting practically monthly since 1973, together with the BBC staff, educational technologists, technicians and researchers who comprise the full Course Team, has welded us into a collective teaching group. In such a team, every assertion about what should be included and how it should be approached has to meet the challenge and critical assessment of our colleagues. We have been able to seek the advice of expert assessors outside the team to comment on various sections of the Course during its development and to call on a panel of students to assess the drafts of the Units for their success as teaching documents, so as to point to areas of potential obscurity or theoretical or factual overload. It has been particularly instructive and we believe useful to have had, for instance, biometricians confronted with developmental geneticists and being forced to convince each other—and the rest of us—of the case for including their respective approaches in a Course in which every minute of the students' time is precious and academic pretentiousness or hobbyhorses cannot intrude. One result, incidentally, has been a considerable rethinking of the amount of mathematics that is essential to an understanding of biometry at this level and a series of experiments in ways of presenting it which we have been able to test with a number of volunteer students.

The Course itself

The Course itself, seen as a linear sequence (although each of its Units may be used separately) begins by asking: What is genetics? What sorts of questions do geneticists ask and how do they obtain the answers? Not surprisingly it goes back to Mendel, and includes a reprint of a translation of his original (1865) paper at this point. There follow four Units on chromosomes and genes; recombination; linkage and maps; and chromosome organisation and changes. The next three Units cover molecular genetics, cytoplasmic inheritance and developmental genetics and there are five Units on the analysis of populations, biometry, plant



and animal breeding and evolutionary and ecological genetics. The final two Units focus on human genetics, and confront some of the major currently controversial issues in human genetics (for instance the question of whether the biometrical approach has anything to contribute to the analysis of group differences in IQ score, the XYY programme and the prospect of genetic engineering). The main teaching sequence is accompanied by a parallel Unit which sets present-day genetic theories into the context of their history and social relations (not forgetting past controversies like that around eugenics and social Darwinism). There is also a specially prepared statistics Unit. All the texts are designed to be self-instructional, with opportunities for the students to assess their own progress by tackling problems *en route* through the Units (OU students will also have a series of programmed tutorials through the year).

Integral to the teaching material are the video and audio tapes, which will be transmitted fortnightly by the BBC over the year, beginning this month. The TV includes both studio animations and experimental demonstrations and also film material involving many leading geneticists, both in this country and abroad, in their own labs or in the field. The radio has a varied role, from radio-vision (for example of fine-structure genetic mapping and the derivation of biometric equations) through to historical programmes on the development of phage genetics and the Lysenko episode. Among those taking part in radio or TV programmes (apart from members of the Course Team itself) are Bradshaw, Cain, Crick, Delbrück, Edgar, Lewontin, Levins, Luria, Sager, Sonneborn, Stent, Stern, Suzuki and Watson.

Finally, for OU students there is a "package" of experiments to be done at home which include genetic studies on tomato seedlings, on micro-organisms and *Drosophila*, as well as a set of observations to be made on humans which will be sent back to be analysed centrally for their genetical implications.

For OU students taking the Course this year, it will be just one component of their degree programme. But at the

same time they will be participating in the final stages of a substantial experiment. Throughout the year we will be monitoring their progress, and that of

students at some conventional universities and colleges who are also using the Course or parts of it. For only this will tell us how close we are to our

ultimate objective—a first rate and comprehensive second level genetics teaching Course which can be widely used in Britain and abroad. □

USSR

Environmental protection under state socialism

In the Soviet Union environmental issues have surfaced in recent years as just one consequence of the country's rapid development. The problems seem as intractable there as anywhere else. Vera Rich reports.

ECOLOGICAL problems seem to be as much a concern of the Soviet Union as they are of other industrialised countries. The prospectus for the Five-Year Plan lays considerable stress upon conservation and environmental protection, and the science and technology section includes among its major aims: "to study the scientific principles of the use and conservation of soils, mineral resources, the plant and animal worlds, the air, and water basins. To expand complex investigations of the world's oceans. To effect the further development of methods of forecasting the weather and natural disasters".

Commenting on this programme in the English-language *Moscow News* (No. 1, 1976), Academician Inokentii Gerasimov implies that the problem of conservation, although a real one, arising from rapid urbanisation and industrialisation, is nevertheless qualitatively different from that in capitalist countries: in the Soviet Union, he argues, "there are no social reasons for the irrational utilisation of natural wealth. Environmental protection under socialism, with its planned economy and no privately owned natural resources, becomes an important task of the state. Hence, environmental protection in our country is part of the current and long-term economic plans".

Taken at its face value, Gerasimov's comment would imply that, in the Soviet Union, any ecological hazard would be of a short-term nature only: the result of a temporary imbalance between one part of the Plan and another. Such short-term imbalances do occur, and at a fairly low level of the administrative hierarchy may become the subject of criticism in the media. Last November, for example, Minsk Radio noted that reforestation (to replace planned felling) in Byelorussia was falling behind schedule,

while in the Brest, Minsk and Grodno Oblasts, excessive felling had taken place and the authorities concerned had failed to take the required "essential measures" against fire and timber-poaching. "Anti-social elements"—fish-stealers, litter-droppers, and others who have not yet learned the norms of behaviour proper to a socialist society—present another possible environmental threat. They too are the subject of exhortation in the media, particularly at the local level; on occasion, the state-sponsored Society of the Friends of Nature has been called upon to organise vigilantes to deal with a particular outbreak of "hooliganism".

These, one might conclude from Gerasimov, are the main dangers to the Soviet environment—fairly local and minor disturbances, far down the administrative and social ladder. In fact, the problem is somewhat more complex. The Soviet authorities have in recent years become increasingly aware that their natural resources, though vast, do not constitute the infinity promised to Peter the Great by his geographers. At the Twenty-Fourth Party Congress in 1971, Mr Brezhnev stressed this new approach: "As we take steps to accelerate scientific and technological progress," he said, "we must ensure that this is combined with the rational use of natural resources and should not cause dangerous pollution of air or water, nor exhaust the soil".

This marks a fairly new approach. The early years of the Soviet Union were marked by rapid industrialisation. On posters and cartoons, the smoking factory chimney became the standard symbol for progress. In accordance with Lenin's equation (Communism=Socialism+Electrification), rivers were dammed and spillways built with little, if any concern for the ecological consequences. The writings of Marx dwell mainly on the urban proletariat and barely touch on conservation; and from Lenin's work isolated quotations can be extricated to serve as slogans. Concern was directed more towards utilising than conserving natural resources; of the major theoreticians, only Engels showed concern with the long-term outcome of remodelling the environment. For many reasons, per-

haps ideological as well as practical, the relevant passages seemed to make little impression on the planners. To turn rivers aside from their courses, to plant orchards where there once were deserts, to carpet the barren steppe with horizon-to-horizon grain-fields—such achievements, as well as bringing immediate economic benefits, would demonstrate at home and abroad the intrinsic superiority of the Soviet system of planning.

In the 1920s, of course, in spite of industrial expansion during the last decades of Tsarism, the vast majority of the Soviet Union was still in the state of a country emerging from feudalism, and a system of tied serfdom remained within living memory. Thus, after some elementary laws were passed—the nationalisation of land, the prohibition of fish poaching, the establishment of certain national parks and health resorts—little attention was given to ecological problems: the drive to take the country into the twentieth century outweighed all other considerations. Only two major pieces of conservation legislation were passed under Stalin. These dealt with the establishment of "shelter belts" of trees to prevent erosion (1948) and the control of air pollution (1949), and the first remained something of a dead letter.

Only during the late 1950s, under Khrushchev's policy of opening up undeveloped areas, were a large number of laws passed on conservation and the environment, reflecting a growing awareness of the need for a coherent policy in this field. In many cases, the laws passed at that time set extremely high standards, as though they were intended as "socialist ideals" rather than practical policies: in certain cases virtually the same law was promulgated several times, suggesting considerable difficulties in implementation. Nevertheless, the laws were published in the press and became common knowledge. In the atmosphere of "the thaw", concerned individuals began to voice protests against the most flagrant infringements either officially, or, later, through the *samizdat* literature. Furthermore, the growing international concern with conservation impelled the Soviet authorities, if only for prestige reasons, to show that the homeland of socialism was not lagging behind in its concern about a universally pressing problem. It is now clear that many of the "developers" were, by present standards, carried too far by their zeal. Many recent Soviet "achievements" in

conservation are, in fact, motivated by the need to clean up the mess of a former generation.

The case of Lake Baikal

A classic example of this is the case of Lake Baikal. This lake, the deepest in the world, has long been famous for its pure water and its extensive flora and fauna, with 708 unique types of living organisms, including freshwater seals. In *Moscow News* Gerasimov announced proudly, "It can be definitely said now that Lake Baikal will not die of pollution, as have many other lakes in the world". Under the Tsars, virtually the sole contact between Lake Baikal and the world of industry was the Trans-Siberian railway which skirted part of its southern shore. Inevitably, perhaps, the forests of the Baikal region came to be utilised, and the tributaries of the lake used for rafting timber prior to its further shipment down the Angara, Baikal's sole outlet. But the construction of cellulose and pulp mills in the unique habitat of Lake Baikal seemed odd in a system claiming to use natural resources "rationally" for the good of present and future generations.

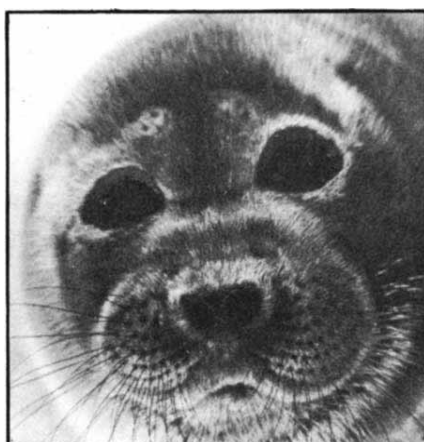
The plans for the mills were approved in 1957, but it was not until 1962 that the first authoritative protests began with an article in *Komsomolskaya Pravda* by the Director of the Limnological Institute of the Siberian Branch of the Soviet Academy of Sciences. Over the following decade, numerous similar articles were published, notably in *Literaturnaya Gazeta* and *Priroda*; Gerasimov himself was prominent in the campaign. Since campaigns of this nature in the State press (as opposed to the *samizdat* network) can only take place with tacit approval from Government bodies involved, there was either a high-level interdepartmental dispute over Lake Baikal or at least an effort to show official concern.

The indications are that there was opposition from the Academy of Sciences, the Geographical Society of the USSR, and the Expert Commission for the Coordination of Scientific Research. Nevertheless, under the auspices of the Ministry for Timber Production, the mills were built and began operation, discharging effluent into the lake. Even when effluent treatment installations were fully operational, the water discharged was yellowish and barely potable, and by no means comparable in quality with the original lake water. The only sound "economic" reason ever offered for the siting of the mills was the exceptional purity of the Baikal water—a purity needed for the production of certain high-quality products. As early as 1965, however, it was observed that water from the

effluent outfall was being carried back by the current to the "pure" water inlet of the mills (*Priroda*, No. 11, 1965). Consequently it was necessary to process the intake water before use. This demanded the installation of expensive pre-treatment equipment the authorities had hoped to avoid by using Baikal water in the first place.

During the last five-year plan, considerable attention was devoted to the problem of the Baikal habitat. New legislation with Party backing was introduced in September 1971, and a special "emergency charter" was approved by the Ministry of Land Reclamation and Water Economy in November 1974. Timber-felling was forbidden within a radius of 50 km of the lake, and the tributaries of Baikal have been cleared of the sunken timber which might have absorbed much of the oxygen from the water and covered fish-breeding grounds; "very costly" outfall treatment plant has been installed at the cellulose mills, and white-fish hatcheries have been introduced to replenish depleted stocks.

Lake Baikal has always been of keen interest to ecologists throughout the world, but more recently it has attracted increased attention because of the construction of the Baikal-Amur Railway. Ecological restrictions relating to this at times verge on the absurd (workers must not spray mosquitoes with insecticide) or over-zealous (devastated areas from natural forest fires on the route are to be planted with seedlings, rather than left to regenerate naturally). But if all the recommendations are carried out, the future of Baikal is certainly assured, and the lake and its environs could well become an ecological show-place. The decision to admit American scientists to observe and participate in research on the lake suggests that already the worst hazards have been dealt with, which is gratifying. But the general question remains whether state ownership of resources entails their best use and protection.



Freshwater seal from Lake Baikal

CANADA

SINCE the beginning of the year the outlook for Canadian science and technology for 1976 has not seemed a particularly optimistic one. The Ministry of State for Science and Technology, contrary to some predictions, did manage to survive the federal government's anti-inflation programme which killed off a number of other federal initiatives such as Information Canada, the Company of Young Canadians and Opportunities for Youth, and reduced others, such as the Local Initiatives Program. But the programme still means less money for science and technology generally.

Specifically, the Industrial Research and Development Incentives Act, which has provided between \$20 million and \$30 million a year for industrial research, will be repealed; reductions will be made in the Programme for Advancement of Industrial Technology and the Defence Industry Productivity Programme; and medical and other scientific research grants will be frozen.

The cuts were made in an attempt to prove to Canadians that the federal government was serious about fighting inflation, and that it intended to set an example. But since then the Prime Minister has gone further, telling the Canadian people that the anti-inflation measures are in fact attempts to control an economy that proved itself unable to work as a free market system—a remark that produced angry responses, including a call for an election by a former Progressive Conservative Cabinet minister.

Altogether, the government said it would cut \$1,500 million from its future spending plans. The loss to scientific research funds was estimated at \$14.8 million and to industrial incentives at \$8 million. These are losses that the scientific community mostly regards as insupportable, in the light of the federal government's recent policies and the impact of inflation.

In a letter to the editor of the *Toronto Globe and Mail*, John Polanyi, Professor of Chemistry at the University of Toronto, pointed out that the total funds available to the National Research Council (NRC), the chief funding body for fundamental research in Canada, will have increased at an average rate of only 2.5% a year from 1969 to 1977, while the cost of doing research during the same period had increased by 100%.

The NRC grants committees, on which he has served, "are quite unable to keep existing research projects of high promise moving ahead, while at the same time giving a genuine opportunity to the scientists of tomorrow to prove their mettle". And, he went on,

"our neglect of science is something that sets us clearly apart from countries with which we might reasonably compare ourselves. In the United States, in France, in Germany, even in beleaguered Britain, the support of basic science has roughly kept pace with inflation. Only in Canada has inflation been used, year after year, as a device for diminishing the nation's investment in this fundamental activity."

In a letter to the same newspaper, Dr Gordon Forstner said that in medical research "somewhere between 150 and 300 technicians will lose their jobs, and a programme which has been deflated steadily during the last five years will be shattered."

● In such a climate, it was with mixed feelings that some Canadians heard that the heavy water plant at Glace Bay, Nova Scotia, was starting up and would shortly produce heavy water not only for nuclear reactors in Ontario and Quebec but also for those in Britain, Argentina and South Korea. Although heavy water is vital to Canada's CANDU reactors (in which it acts as coolant and moderator), the Glace Bay plant has been one of the most disastrous undertakings of an otherwise successful nuclear programme.

In 1964, the province of Nova Scotia brought in a US nuclear scientist, Jerome Spevack, to design the Glace Bay plant. There were difficulties from the outset. Completion was delayed from 1966 to 1967, then again to 1969. Technical difficulties occurred as a result of using salt water from the nearby Atlantic Ocean in the process, instead of fresh water. Finally, in 1970, inspectors discovered that the salt water had corroded the pipes, and a \$30 million repair was needed.

By that time, Spevack's company, Deuterium of Canada Ltd, had spent \$100 million on the plant, all of it public money, because Nova Scotia was the major shareholder and provided the finance. The province had bought out Spevack's interest for \$3 million in 1966, and taken full control in 1969. In 1971, the federal government provided the funds for the plant's purchase by Atomic Energy of Canada Ltd (AECL), and spent another \$130 million on what has been almost a complete reconstruction.

Eventually, the plant is expected to become self-sustaining and make enough money to pay back AECL's investment. The Nova Scotia government is to get the plant back after AECL has recouped its investment, but the province plans them to sell it back once more to AECL—finally washing its hands, as it were, of the whole affair.

David Spurgeon

Ottawa

USA

Alternative technologies urged in pesticide report

Carried to excess, the best things may do more harm than good. Colin Norman reports from Washington on a study of pesticides.

A COMMITTEE of the National Academy of Sciences has warned, in a mammoth study published last week, that unless alternatives to conventional chemical pesticides are swiftly developed and adopted, agricultural production in the United States could soon begin to suffer. The committee bases that conclusion on the fact that several potent products have been either severely restricted or banned entirely because of environmental and health hazards, and also the fact that several pests are developing resistance to the poisons which are sprayed on them in copious quantities each year.

The study, which took more than three years to complete and which runs to five massive volumes, is an attempt to assess the current state of the art in pest control and to pinpoint some of the problems which lie ahead. In the course of its analysis, the committee has questioned the costs of some government regulations, criticised current pest control practices, cast doubt on the value of some of the Department of Agriculture's most ambitious and costly efforts to eliminate particular insect species and, by implication, criticised the federal government's agricultural research efforts.

The basic theme running through the huge tome is that although chemical pesticides have served agriculture—and, for that matter public health pro-

grammes—very well, the problems of declining effectiveness "warrant substantial expansion of present efforts to promote alternative technologies, including integrated pest management". The chairman of the committee, Dr Donald Kennedy, professor of physiology and zoology at Stanford, said last week, for example, that genetic resistance to toxic pesticides is growing at an "alarming rate", and he suggested that unless effective alternatives are developed, some agricultural land could conceivably be taken out of production.

As for specific alternatives, the committee notes that so-called 'third generation' compounds, which affect hormonal development or reproductive processes in insects, have some desirable qualities, but it suggests that "there is reason to be pessimistic about the prospects for controlling major crop pests with these compounds." One potential problem with third generation agents is that resistance is likely to develop to them, the committee states.

Insect control by pathogens, such as baculoviruses, is especially promising, but the committee cautions that large-scale use of such agents will require improved methods for culturing insect host tissues. Moreover, the development of new agents will require some advances in basic research. The use of genetic techniques, such as breeding resistance to pests into crop plants, and introducing genetically modified pests into the environment, are also promising, but again the committee



Crop-dusting: must an alternative be found? (Photo: Popperfoto)

cautions that there are potential problems facing widespread application of some of the techniques. The use of sterile male insects to reduce reproduction in a target population, for example, is effective only when the population has been reduced by other means. Moreover, since potential markets for sterile male insects are likely to remain small, there is little commercial incentive for private firms to get into the business of producing them.

Thus, the general message is that although various alternatives are promising, there is no magic insect zapper", as Kennedy put it, to replace chemical poisons in the near future. "The task of pest control over the next 10 years will almost certainly become larger rather than smaller", the committee concludes. It therefore recommends that research efforts be stepped up, and in particular that basic research on organisms and ecosystems be given more attention by various government agencies.

As for efforts designed to eradicate individual pest species entirely, the committee warns that such schemes have limited chances of success and frequently turn out to be inordinately expensive. In particular, the committee recommends against a programme,

strongly urged by some scientists in the US Department of Agriculture, which is designed to eradicate the boll weevil from the cotton fields of the southern United States. The scheme, which would employ a variety of pest control techniques, would cost about \$1,000 million. Although extensive trials conducted in 1972 indicate that the programme would have a good chance of success, the committee suggests that there is considerable doubt that the boll weevil could be eradicated entirely. The committee cautions that if the programme fails, it may endanger public confidence in the alternative methods of pest control which would be used.

One of the problems which the committee encountered in its research was an appalling scarcity of data on pesticide use in the United States. "The pest control enterprise places a billion pounds of toxic materials into the environment each year", the committee states, "but it is considered 'normal' for us to have only the vaguest idea of how much each compound was used where, and then only after a decade lag". It therefore urges that much more effort be put into monitoring pesticide use and that chemical companies be required to report their production and sales figures to the federal government. □

OSETP progress

A longstanding desire of the scientific community in the United States came a step closer to reality last week when the Senate unanimously approved a bill to re-establish a science policy office in the White House. The bill is similar in many respects to a version passed by the House last November, but there are a few important differences which must be ironed out before the measure is sent to President Ford for his signature. The Senate bill would establish an Office of Science, Engineering, and Technology Policy in the White House, headed by a Director who would also be the President's Science Adviser. Unlike the House bill, the Senate version specifies that the director of OSETP would sit on the powerful Domestic Council and be a statutory adviser to the National Security Council. The Senate version would also set up a programme designed to strengthen science policy arrangements at the state level, and to provide grants to states for the application of technology to various pressing domestic problems. It is now expected that the bill will be ready for Mr Ford's signature by the end of March.

Triple resignation

THE embattled nuclear power industry in the United States suffered a potentially severe political setback last week when three senior engineers in the reactor division of the General Electric Company (GE) quit their jobs and announced that they will campaign for anti-nuclear groups. They said in their letters of resignation that they have become so concerned about questions of reactor safety, proliferation of nuclear weapons, and radioactive waste disposal that they can no longer work for the industry and keep their doubts to themselves.

Each had spent his entire professional career working for GE and, until they quit on February 2, all three were employed at the company's facility in San Jose, California. Although they have raised no new issues, their espousal of the anti-nuclear cause will clearly have substantial impact on public concern about the hazards associated with nuclear power.

That impact is likely to be most keenly felt in California where, on June 6, voters will determine the future for nuclear power in that state. All three have announced that they will campaign heavily in support of a

proposition, to be put to a state-wide vote during the California Presidential primary election, which would almost certainly halt construction of nuclear power plants in California and eventually lead to shut down of existing plants there.

News of the resignations prompted swift reactions in Washington. Congressmen and Senators who have previously expressed concern about nuclear power issued statements publicising the development; Senator John O. Pastore, the chairman of the powerful Joint Committee on Atomic Energy, has tentatively scheduled a committee hearing on February 18 at which the three engineers will testify; and the head of the Nuclear Regulatory Commission, William A. Anders, met with them last week to hear their concerns.

At a press conference called by the Union of Concerned Scientists, an outspoken anti-nuclear organisation, the three engineers said last week that no single event prompted their departure from the world's largest manufacturer of nuclear equipment. All three said that their concerns have been growing for some time, and they felt that the nuclear industry has been seriously downplaying the risks

associated with nuclear technology.

The three engineers are Dale G. Bridenbaugh, formerly Manager of Performance Evaluation and Improvement, who has headed a special project to assess the adequacy of the primary containment vessels of GE's nuclear reactors; Gregory C. Minor, former Manager of Advanced Control and Instrumentation, who has been responsible for design of safety systems and control room instruments; and Richard B. Hubbard, former Manager of Quality Assurance, who has been responsible for ensuring that GE's reactor equipment meets federal quality standards.

Though all three said they reached their decisions to leave the nuclear industry independently, Minor's letter of resignation probably sums up their feelings with the statement that he is "convinced that the reactors, the nuclear fuel cycle, and waste storage systems are not safe. We cannot prevent major accidents or acts of sabotage. I fear that continued nuclear proliferation will quickly consume the limited uranium supply and force us into a plutonium-based fuel economy with even greater dangers of genetic damage and terrorist or weapons activity".

ISRAEL

Harnessing science and industry

As the need to coordinate the work of science and industry even more closely becomes increasingly apparent to the recession-bound developed countries, Nechemia Meyers reports from Israel on the debate taking place there.

"PLANS for increasing the scientific potential of Israeli industry were ready four or five years ago, but no one seemed to be in a hurry to implement them. Now, under the pressure of our worsening economic situation, people in science, government and industry are beginning to think and act much more realistically". So says Dr Eliezer Tal, Director of Israel's National Council for Research and Development.

This new realism comes none too soon, for it will be required if solutions are to be found to the problems Israel now faces. First, Israeli industry must turn out more goods that can compete on the world market and, as Dr Tal points out, these will not come from the labour-intensive enterprises that were opened with such fanfare in the 1950s and 1960s. "In view of Israel's European-level wage structure," Tal argues, "she must concentrate on the development of high-technology products equivalent in price and—more important—in quality with those coming from the USA, western Europe and Japan."

Another problem intimately linked with this is the one of finding jobs for science graduates. The country's seven institutions of higher learning are fully staffed, and with budgets for higher education on the decline—they went down 30% in real terms during the past two years and will drop still further this year—some lecturers may even be dismissed. Only a more scientifically oriented industrial sector could, at least theoretically, absorb thousands of 'surplus' science graduates, products of local universities and immigration alike.

A controversial approach to achieving both these goals was recently offered by Richard S. Rosenbloom, Professor of Business Administration at Harvard and, of late, Visiting Professor at the Hebrew University of Jerusalem. The primary problem, as he sees it, is not that Israel spends too little on research and development. On the contrary, the country devotes a larger share of its economic resources to those ends, about 2.4% of its GNP, than any Western nation except the

USA. Even when military research and development is discounted, Israel's expenditure, 1.8% of its GNP, remains greater than the total effort of Belgium and Denmark, and about the same, per capita, as spending on civilian research in Sweden and France, although less than in the USA, the UK and the Netherlands. What disturbs Rosenbloom is that only 14.6% of Israel's civilian research and development is carried out in the business sector, as compared to 50% in Denmark and 80% in Switzerland. And the universities, where most of the other research and development is carried out, are not geared to matching technological opportunity with market needs.

What is the answer? Professor Rosenbloom has a revolutionary suggestion: the creation of an entity he calls "Israel Inc.", which would provide a countrywide framework for the innovative exploitation of technology. Israel Inc., although prepared to consider any area of research and development, would probably concentrate its attention on those where existing achievements indicate that meaningful results are most likely to be achieved, including chemicals, drugs, aircraft, industrial machinery, electronics and instrumentation.

Realising that the idea may sound "strange and impractical", Rosenbloom cites as a possible parallel the experience of General Electric (GE). GE, a highly diversified company which employs 307,000 people (as compared to the 250,000 employed by all industrial companies in Israel), has sales roughly four times as large as those of Israeli industry as a whole, and, with 700 identifiable product lines, covers almost as wide a spectrum as all manufacturing companies here put together. GE might serve as a model, Rosenbloom says, because it is not a single monolithic body, but rather a collection of quasi-independent operating units which are, at the same time, subject to certain strong influences from a central organisation.

The first step towards "Israel Inc." would be the creation, under government auspices, of a National Council for Industrial Technology, which would include the heads of all large science-oriented companies, representatives of some of the smaller companies, academics from pertinent university branches and senior government officials. Sub-groups of the council would deal with such topics as market projections, technological

evaluations, manpower, funding and so on.

Next on the Rosenbloom agenda would be the establishment of an Authority for Industrial Technology, managed by the council and owned jointly by industry and government. The authority would operate its own national research and development laboratory and, at the same time, stimulate contact between research and development establishments in various sectors of the economy. Engineers and scientists employed by the authority would not only work in its laboratory, but also be sent out to other research and development centres and to universities. This system of rotation, Rosenbloom believes, would serve to break down the barriers that now hamper the flow of knowledge into industrial innovation and cut off contact between researchers and the markets in which their knowledge could be exploited.

Dr Tal agrees with Professor Rosenbloom on many points, including the advisability of merging the welter of small companies which now exist into large industrial units where serious research and development becomes possible, and the need to link industrial research with industrial policy. The Israel Government, Dr Tal declares, must decide on specific industries to promote, rather than encouraging investment in a wide range of enterprises. He would, however, shy away from the establishment of something like "Israel Inc.", which "might stifle initiative rather than promote it."

Attitudes inside industry also had to change. All large American firms, Tal points out, have a Vice President for research and development who plays a key role in policy decisions. This is far from being the case in Israel where, for example, it was only a few weeks ago that one of the country's largest companies even appointed a top level adviser on technology. Vice Presidents are still undreamed of.

A Swiss-born chemist, Rene Bloch, who heads a small but rapidly developing company engaged in work on synthetic membranes, has his own suggestion for the promotion of industrially oriented research. "To attract our most brilliant scientific minds to industry, we must give them more money and, particularly, more prestige than their university colleagues." As a symbolic first step, Dr Bloch suggests that the country's major national scientific awards—the Israel Prize and the Rothschild Prize—should henceforth go not to "ivory-tower types whose research pays off in papers", but to applied scientists whose research pays off in dollars. □

IN BRIEF

Concorde verdict

The US Secretary for Transportation's 16-month probationary clearance for Concorde, which immediately inspired the Environmental Defense Fund to apply to the District Court of Appeals for a review of the verdict, is still expected to receive Presidential support, despite hostile Congressional opposition. A decision on access from the airport authorities involved is now awaited: the Port Authority of New York and New Jersey, responsible for Kennedy airport, is reportedly anti-Concorde, but Washington's Dulles Airport is Federal controlled.

JET set?

Strong demands for a final decision on the siting of the Joint European Torus (JET) are likely when the EEC council of research ministers meets at the end of the month, and Britain looks like losing not only the battle to have the £70 millions fusion project sited at

Culham but possibly the 40 scientists engaged on preliminary work there as well—with Britain apparently isolated in its opposition to the EEC Commission's recommendation that the project should go to Ispra in Italy, the United States is rumoured to have started moves to collect Culham talent for its own fusion research programme.

AGRs start

Years behind schedule, two of Britain's highly expensive 1,250 MW advanced gas-cooled reactors (AGRs), Hinkley Point B in Somerset and Hunterston B in Ayrshire, last week made their first contributions to the national energy supply. Hinkley, now with an estimated capital cost of about £159 millions, generated only a little electricity before being stopped, pending further tests, but both are expected to build up to full power over the next few months.

Mediterranean clean-up

Experts from Mediterranean states,

meeting for two weeks in Barcelona under the auspices of the United Nations Environment Programme to discuss measures to clean up and prevent pollution of the Mediterranean, have received the text of a draft treaty embracing pollution from rivers and coastal establishments and exploitation of the sea bed, as well as dumping by ships and aircraft.

Indian oil

The Indian Government is to increase investment in oil exploration by a third to £200 millions as part of a bid to boost crude oil production. The target is self-sufficiency by the early 1980s, by which time the annual requirement is expected to be around 30 million tonnes. Though land-based wells at present produce less than a quarter of that figure, seismic surveys indicate potentially rich reserves in the offshore continental shelf. Optimistic interpretations even suggest that India could be an oil exporter within a decade.

THE toxicity, stability and pervasiveness of polychlorinated biphenyls (PCBs) as uncontrolled contaminants are known from a series of investigations that started in 1966, but very little has been done about it. Poisoning occurred in Japan in 1968 from eating rice oil containing PCBs. Clinical effects reported included stillbirths, undersized infants, bone and joint deformities and neurological disorders. Wholesale poisoning of chickens took place when PCBs leaked into fishmeal during processing. The contaminated feed produced low hatchability, and many newly-hatched chicks died. PCBs are distributed widely in major waterways in the USA; their presence led the Food and Drug Administration to warn against eating Lake Michigan fish, and the US Fish and Wildlife Service to call recently for eliminating all sources of PCBs in the environment within three years.

Apparently PCBs have escaped bans because they do not kill insects. They are frequently compared with DDT, which they resemble somewhat in chemical structure, and they can be mistaken for DDT in chromatography, but the resemblance ends there. PCBs are far more stable and more toxic to vertebrate organisms than DDT, which is broken down by enzyme systems that do not change PCBs. PCBs were in North Atlantic surface water at about 20 parts per 10^{12} as compared with less than one part for DDT, even though the fall-out of DDT was estimated as twice that of PCBs. The Environmental

Protection Agency (EPA) is conducting a vendetta against DDT and other chlorinated hydrocarbon insecticides, but has soft-pedalled PCBs—perhaps because of pressure and influence on the EPA by the Environ-

DDT: bystander or participant?**THOMAS H. JUKES**

mental Defense Fund (EDF), whose head scientist engagingly revealed EDF's motivation as follows: "If the environmentalists win on DDT, they will achieve, and probably retain in other environmental issues, a level of authority they have never had before . . . In a sense, then, much more is at stake than DDT".

The toxic effects of PCBs and mercury on wild birds may have provided a convenient weapon for clobbering

DDT. However, Scott and his collaborators at Cornell University simultaneously compared the effects of PCBs, DDT and methyl mercury on laying hens. Hatchability was reduced at 8 weeks from 92% in the controls to 50% with 10 p.p.m. of PCBs and to 2.4% with 20 p.p.m. DDT had no effect on egg production or hatchability at the highest level fed (100 p.p.m.), and increased the breaking strength of eggshells on a low-calcium diet. Methyl mercury, 10 p.p.m., adversely affected egg production, egg-shell strength, fertility, and hatchability, and it increased morbidity and mortality. Adverse effects attributed to DDT, especially on eagles, have been reported in regions contaminated with mercury. The Cornell workers noted that many reports relating reproductive declines in wild birds to DDT and DDE were based on analyses that did not distinguish DDT from PCBs, and that some investigators had tried to reinterpret results in terms of PCBs.

DDT has never been recorded as having poisoned human beings through the food supply of by industrial exposure. Scott *et al.* say that "condemnation by correlation". in the case of DDT, "may have been downright dangerous to public health." In contrast to pesticides, whose transport and use are publicised, monitored and regulated, PCBs are unrestricted. They have been dumped out of discarded electrical capacitors and transformers, and put on roads to settle dust. The dust is now being stirred up again.

correspondence

Closure in Belgium?

SIR,—Due to a political decision, a research institute I head is about to be closed (*Institut de Recherche Interdisciplinaire en Biologie Humaine et Nucléaire*) and another to be severely handicapped (*Département de Biologie Moléculaire*, under Professor J. Brachet) at the University of Brussels. In a European country where the stability of employment is high and the turnover rate of personnel very low, this situation is highly unusual.

The Institute was created in 1963 to develop, in connection with clinical research units and with a sister group in Pisa (Italy), the use of radioisotopes in medical research and medicine. Since then, it has slowly evolved into what is now the *Institut de Recherche Interdisciplinaire* of the *Université Libre de Bruxelles*, a lab where physicists, chemists, biologists and MDs collaborate in biomedical research on many subjects (thyroid metabolism, cancer, cyclic nucleotides, lung physiology, theoretical biology, etc.).

Since its creation, the group has been subsidised by Euratom and the Universities of Brussels and Pisa. In 1969, Belgium did not participate any more in the Euratom programme of Nuclear Medicine. At that time, the government decided to support and create new research groups, "Actions Concertées", in the universities which would, like the Medical Research Council research units in Great Britain and the National Institutes of Health clinical research centres in the US, be catalysts for university research. This has worked well since then. The groups of Brussels were the first to be created and thus the first to arrive at the end of their five year contract.

This year, the government developed a new framework for research in which the whole structure of Belgian research is changed. This project has been criticised. One of its features is the concept of subsidising university research in proportion to the number of students. The government has agreed that the groups would fit in well in this new programme, but it has tied the granting of finance to the "Actions Concertées" to the approval of its programme. As the new project is now delayed, and as the contract is coming to its end, no financial support is planned for 1976. Thus the group is now, because of a political squabble, left "in the air".

Most of the permanent staff was not paid at the end of December. Thus in a few weeks the group might be disbanded. This situation has been much discussed in the Belgian press. The Minister insists that the Universities should take over temporarily, but the hard pressed university is financially and legally unable to do it. It is already badly burnt by a new "loi-programme" on finances, which includes a new subsidisation scheme for universities and which caused much turmoil and student demonstrations in the country in 1975.

Yours faithfully,

(Professor) J. E. DUMONT

Institut de Recherche Interdisciplinaire en Biologie Humaine et Nucléaire, Université Libre de Bruxelles, Belgium

Crisis in Italian universities

SIR,—I was impressed by the objectivity and completeness of Gillian Boucher's article (November 20, page 190). The causes of the Italian university crisis do not require further elucidation, still less comments like those of Giacomo Morpurgo (December 11, page 475). Morpurgo's letter, if published in an Italian magazine and not in an authoritative and widely circulated scientific journal such as *Nature*, would not deserve mention. His statements are ridiculous and untrue.

It is absurd to assert that the "intended aim" of the recent laws was to assure a permanent position for a large number of leftists "disposed to aggressive political activity". Morpurgo knows that these laws were promulgated by a Christian Democrat government which has ruled the country for thirty years.

After the results of the June 15 elections nobody should be surprised that the majority of university teachers are left wing. The intellectual class in Italy (with the exception of a few people like Morpurgo) was leftist long before June 15.

It is true that departments are invaded less frequently now than a few years ago. But this phenomenon is not confined to Italy. In every country student dissent is weaker than it was in 1968, for reasons much more serious than those invented by Morpurgo.

The recent laws have given tenure to a large number of university teachers and researchers whose positions had been precarious for a long time pre-

viously. The laws have repaired old injustices. If many teachers do not deserve their present positions, that is the fault of the University "baroni" who, by keeping their staff in menial positions for their own convenience, have given them the right to exploit the new laws. One of the privileges of the full professors was to create whatever positions they wanted and to assign them to whoever they liked for as long as they liked. If today the Department of Physics in the University of Genoa has more teachers than students, that, too, is the fault of Professor Morpurgo and his colleagues.

Yours faithfully,

FRANCESCO GHIRETTI

ANNA GHIRETTI-MAGALDI

Institute of Animal Biology, University of Padua, Italy

Water to the Dead Sea

SIR,—While it would be possible to generate electric power (November 6, page 9) from the flow of seawater into any depression lying below sea level—the Dead Sea (surface at -390 m), the Qattara Depression (-138 m), or possibly even the Caspian Sea (-28 m)—there is one thermodynamic possibility that applies to the Dead Sea alone. It is possible to deliver fresh water to the valley of the Dead Sea by a spontaneous process using a system with no moving parts.

The osmotic pressure of seawater is about 25 bar, and this pressure is reached with a head of 250 m of water. Any greater pressure would cause fresh water to flow through an osmotic membrane such as those used for desalination by reverse osmosis. Fresh water could be delivered to the valley of the Dead Sea not much below -250 m. This water could then be used to irrigate a substantial area, particularly around the mouth of the Jordan River.

To use the flow of seawater into the Dead Sea exclusively for the generation of electric power is to neglect that what the desert most needs is fresh water. There is no reason, however, why the two projects cannot be combined, with fresh water being obtained at negligible added capital cost over that for electric power alone.

Yours faithfully,

G. S. KELI

Y. LUPIN

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news and views

Superfluid ^3He : magnetic pendula

from P. V. E. McClintock

OBSERVATIONS at La Jolla of nonlinear magnetic ringing seem to have demonstrated the existence of significant deviations from the predictions of "simple pendulum" models of dynamic magnetism in superfluid ^3He . The experiments, by Webb, Sager and Wheatley (*Phys. Rev. Lett.*, **35**, 1010; 1975) can, however, be regarded as confirming the general correctness of the by now well-accepted pairing theory of the superfluidity.

In the picture of superfluid ^3He which has emerged as a result of the last three years' intensive experimental and theoretical activity, liquid ^3He -A, which is the stable phase for temperatures near 2.4 mK and pressures above 22 bar, can be considered as a mixture of three separate but interpenetrating components: two magnetically active superfluids capable of exhibiting non-dissipative flow, and a normal fluid component. One superfluid consists of pairs of atoms whose nuclei both tend to point parallel to a magnetic field (the up-spin superfluid) while the other is made up of pairs whose nuclei both tend to be anti-parallel to the field (the down-spin superfluid). There are no pairs with opposed nuclear spins in the A-phase. The two superfluids behave almost independently of each other, being only weakly coupled by way of the nuclear dipole interaction. The coupling is, however, sufficient to allow pairs of atoms to pass from one superfluid to the other so that an equilibrium between their respective populations can be maintained.

Very interesting phenomena follow a small disturbance of this equilibrium by, for example, suddenly changing the magnitude of the applied magnetic field by a small increment: this slightly alters the chemical potential of the up-spin relative to the down-spin superfluid, and thus requires a transfer of pairs between them in order to establish a new equilibrium between their populations.

A striking consequence of Leggett's equations (*Am. Phys.*, **85**, 11; 1974) is that such a flow should be oscillatory in character. In fact, as both Leggett

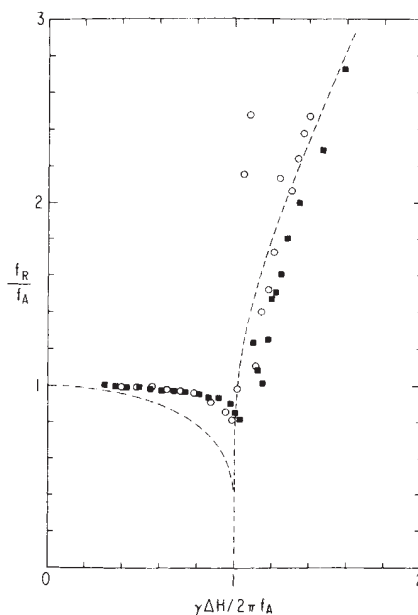
and also Maki and Tsuneto (*Prog. theor. Phys.*, **52**, 773; 1974) have pointed out, there is a remarkably close analogy with the a.c. Josephson effect, which involves an oscillatory transfer of pairs of electrons between two weakly coupled pieces of superconductor. In ^3He , unlike the superconducting case, the two weakly coupled superfluids are not, of course, physically separated, being on the contrary completely interpenetrating. Nonetheless, the physics of the two situations, and the equations that govern them, are extremely similar: as in the case of the Josephson effect, it turns out that the equation which describes the flow of pairs between the up-spin and down-spin superfluids is formally identical with the equation describing the motion under gravity of a simple pendulum. As the result of small disturbances from equilibrium, one therefore expects that the system will oscillate with a characteristic frequency which is independent of amplitude: this internal Josephson effect or (as Richardson very reasonably suggested in his invited address to the LT14 conference) "Leggett effect" has been observed experimentally in a number of laboratories.

Larger disturbances from equilibrium should show non-linearities of precisely the same form as are exhibited by a simple pendulum. For example, one would expect a decrease in frequency at large amplitudes; and, for very large amplitudes, a frequency which is almost independent of potential energy, no matter whether this corresponds to the nuclear dipolar coupling in ^3He or to the gravitational potential energy of the simple pendulum. The latter situation corresponds to the pendulum receiving such a substantial initial push that it goes "over the top" and rotates freely in the vertical plane. It is nonlinear effects of this nature which have been studied in some detail at La Jolla.

One of their experiments involved cooling some liquid ^3He to just below the transition temperature T_c of 2.4 mK, under a pressure of 22 bar. The cell was a long rectangular cavity

with a cross section of 1 mm $\times$ 10 mm, and a static magnetic field was applied parallel to the longest walls: this approximated to an ideal geometry in which the highly anisotropic superfluid ^3He -A should have taken up a well-defined texture. A small incremental change ΔH in the magnetic field was made and a suitably positioned sensing coil was then used to detect the resultant oscillations (ringing) in the magnetisation of the liquid.

As expected on the basis of preliminary experiments and in the light of the theory, the frequency of the ringing was found to depend on the size of ΔH which, in the pendulum analogy, represented the initial impulse given to the pendulum. Their result is shown in



Nonlinear ringing of the magnetisation of superfluid ^3He -A, following a change ΔH in the magnitude of the applied magnetic field (after Webb, Sager and Wheatley, *Phys. Rev. Lett.*, **35**, 1010; 1975). The initial ringing frequency f_R is divided by the characteristic A-phase frequency f_A , and plotted against ΔH which has been normalised such that the anticipated zero in f_R should occur at unity on the abscissa. The dashed line represents a theoretical prediction, and is analogous to the behaviour of a simple pendulum.

Fig. 1. It can be seen that for small ΔH the ringing frequency f_R remains close to the characteristic A-phase frequency f_A . For larger ΔH , f_R decreases, although not so fast as predicted by theory (the dashed line) and passes through a minimum. The latter situation is believed analogous to the pendulum having been given an initial impulse of exactly the right magnitude just to take it to the top of its orbit, where it therefore remains, which is why the theoretical curve indicates zero frequency. The facts that a minimum in f_R is observed rather than a zero, and that the minimum apparently occurs at slightly too large a value of ΔH , may perhaps represent a genuine deficiency of the simple theory. The rapid rise in frequency just beyond the minimum seems, however, to be in reasonable agreement with expectation; and the so-called ringing-up and ringing-down phenomena which were observed close to the minimum constitute a striking verification of the general correctness of the pendulum model: experimentally, it was found that when the initial f_R was just to the left of the minimum in Fig. 1, the frequency increased (rang up) with time, corresponding to the increase in frequency of oscillation of a pendulum when frictional damping processes reduce the amplitude of its swing; and, for an initial f_R just to the right of the minimum, the frequency decreased (rang down) with time, corresponding to the frictional slowing down of a freely rotating pendulum.

Perhaps the physically most significant result of these experiments arises from measurements very close to T_c of the driven mode, corresponding to free rotation of the pendulum, which occurs

for very large ΔH . Under these conditions f_R varies almost linearly with ΔH ; with a constant of proportionality which is independent of the nuclear dipole forces, and is related only to the inertia of the rotating vector which is the analogue of the pendulum. The experimental results have turned out to be in excellent agreement with what would be expected on the basis of the already widely accepted pairing model of superfluidity in ^3He .

The La Jolla group has also performed equivalent experiments for the other, low temperature, superfluid phase, $^3\text{He-B}$. Here, things are much more complicated because a third superfluid component, consisting of pairs of atoms with opposed nuclear spins, is also present. An equivalent pendulum analogy involves consideration of two coupled pendula. Maki and Hu have calculated (*J. Low Temp. Phys.*, **18**, 377; 1975) that there should be two zeros in f_R . The experiments have indeed demonstrated two rather shallow minima, but not occurring at the predicted values of ΔH . However, the ringing up and ringing down phenomena could again be seen; and the frequencies of the driven mode were again in excellent agreement with the pairing model.

The quantitative discrepancies between the La Jolla data and predictions based on the conceptually very attractive pendulum models look real enough, but they will be treated with a certain amount of caution until the experiments have been repeated elsewhere: these measurements are being performed very close to the limits of present technology and the systems under study—magnetically active, anisotropic superfluids—are unique. $\square$

resource partitioning in communities of plants or animals have increased at four times this average rate, with a doubling time of less than 3 years. Our rough estimates indicate that work in theoretical ecology is generating papers at a rate similar to, if not greater than, this empirical work.

In theoretical biology in general, and theoretical ecology in particular, this increase in papers is reflected in an increase in the number of journals—*Biometrika* (born in 1901), *Bulletin of Mathematical Biophysics* (1939), *Biometrics* (1945), *Journal of Theoretical Biology* (1945), *Mathematical Biosciences* (1967), *Theoretical Population Biology* (1970), *Journal of Mathematical Biology* (1974), *SIAM Journal on Applied Mathematics, Part C* (1976). This trend is faster than exponential, and indeed here a naive extrapolation will suggest an infinite number of journals within a few decades. This explosion in number of papers, and perhaps even in information, creates both human and financial problems.

Of these expanding numbers of theoretical papers in ecology, some contain insights of enduring use to theorist and empiricist, while others represent incremental advances in specialised areas. But essentially all those published are worthy of publication in some form. An established hierarchy in the importance of the various journals would be one rational response (and it exists to a certain extent), except that no publisher is keen to accept low place on the totem pole, and many reviewers seem to apply the same standards (sometimes too harsh, sometimes too lenient) to all work, regardless of the journal. This swelling flood of papers in theoretical ecology is exacerbated by traditions that allow mathematicians to present proofs and analysis in exquisite detail. Empiricists, on the other hand, are not allowed the analogous luxury of publishing their field notes. There is much to be said for innovative publishing practices whereby the major mathematical assumptions and conclusions are published, with the full proofs supplied in manuscript form to the referees and available as *samizdat* to those who care to write to the author. This practice could at least halve the bulk and cost of the journals listed above.

A variation of this idea, which has the advantage of conferring greater permanence on the material, is to publish the full paper in microform, and publish a lengthy abstract in conventional journal form. This is a logical outgrowth of the practice of putting journals into microform; for a review of contemporary trends, see Lynden (*Microform Review*, **4**, 15-24; 1975). In a recent survey (News and Com-

The ecology of the ecological literature

from Judith May and Robert M. May

EVERYONE is familiar with the arrant nonsense produced by assuming that the current rate of population growth will continue indefinitely at its present value of about 2% per annum. If such a rate were sustained for around 5,000 years, the globe would be a ball of human protoplasm, expanding into space at the speed of light. This sort of naive extrapolation can be performed for various other quantities, many of which exhibit exponential growth rates faster than that of the human population as a whole. Thus if the rate at which the population of scientists grew in the 1960s were projected, in a mere two to three centuries there would be more scientists than people. Obviously such calculations are

not to be taken literally, but they do dramatise trends: in particular, the estimate above suggests that for the scientific community the growth rates of the 1960s were pathological, and that the slower growth rates of the 1970s are nearer the norm.

One of the basic products of scientists, namely published papers, has also grown exponentially in volume. Derek de Solla Price (in *Little Science, Big Science*, Columbia University Press, 1963) has documented a long term pattern of exponential growth in the number of scientific publications, with a doubling time of 10 to 14 years. Ecologists may rejoice or despair at Schoener's (*Science*, **185**, 27-39; 1974) observation that published studies of

ment in *Microform Review*, 4, 179–182; 1975) of 53 major publishing organisations representing 1,427 journals, roughly one third “expressed themselves in regard to how they would publish in microfiche.” Of these about one half were considering publishing the entire journal in microfiche but with a hard-copy version of contents and abstracts. Many of these publishers were also thinking of publishing the experimental details, appendices and other source and supplementary material only in microfiche and the abstracts or shorter versions of the articles in a paper edition. There are clearly many advantages to a two-tier system, with a conventional journal containing full summaries of the results, and with details published in microform (which could be produced directly from manuscript, saving time and expense). With contemporary techniques paper copies can be supplied on demand from a central microform handling.

Quite apart from the increasing number of journals, the rising production costs for individual journals create problems. One response for many journals has been to levy page charges, and sometimes to discriminate (often by slower publication) against those who cannot pay the charges. Van Valen (*Evolutionary Theory*, 1, 119–130; 1975) has recently made a tentative survey of various kinds of financial discrimination by journals catering to the broad area of ecology and evolution: of the various kinds of subtle and not-so-subtle discrimination he catalogues, the commonest is a mandatory charge for pages in excess of x (where x is typically around 10). Van Valen's figures suggest that of journals published in this general field in the USA, 69 of 112 manifest one or more discriminatory practices. The corresponding figures for Britain are 5 of 41; for the remainder of Europe 23 of 48; for Japan 6 of 8; for Canada 2 of

8. The average cost per 100 pages of the journal to the subscriber is roughly constant for the various countries (with commercial publishers based in Germany tending to be significantly higher), and for the various publishers.

Socially responsible readers of *Nature* will be relieved to know that Van Valen classes it as not discriminatory.

Myelin structure

from N. P. Franks

NEW freeze-fracture studies by Pinto da Silva and Miller (*Proc. natn. Acad. Sci. U.S.A.*, 72, 4046; 1975) have questioned current concepts of myelin structure. Myelin is usually thought to be unlike any other plasma membrane. It has an unusually low protein content (about 20% by weight), very little enzymatic activity (Adams *et al.*, *J. Neurochem.*, 10, 383; 1963) and a lipid composition likely to result in a particularly stable bilayer (O'Brien, *Science*, 147, 1099; 1965). This is all consistent with the principal function of myelin being that of an electrical insulator of nerve axons.

The most widely accepted model of myelin structure is that of a basic lipid bilayer, with most of the protein distributed outside the hydrocarbon region. Support for this picture came from the freeze-fracture results of Branton (*Expl Cell Res.*, 45, 703; 1967), which showed that the fracture faces of frozen myelin were relatively smooth (similar to those seen with lamellar phases of lipids alone) in contrast to the particulate appearance of the fracture faces of other membranes. Since most membranes are thought to fracture within the hydrocarbon region, and since in some cases the particles have been shown to be protein-containing structures, Branton's results were consistent with a bilayer of lipids relatively uninterrupted by protein. More detailed information about the protein distribution has come from the X-ray diffraction work of Caspar and Kirshner (*Nature new Biol.*, 231, 46; 1971). Using an absolute electron density scale, previously established by Blau-rock (*J. molec. Biol.*, 56, 35; 1971), they interpreted their electron density profiles as showing that the concentration of protein in the aqueous spaces between bilayers was 15–20% by volume (possibly higher near the lipid headgroups) with less than 10% by volume at the centre of the hydrocarbon region. The electron density profiles of Caspar and Kirshner impose important constraints upon models of myelin structure and were taken to support the notion that there was little

protein in the hydrocarbon region.

Surprisingly, Pinto da Silva and Miller now report a widespread distribution of particles on the freeze-fracture faces of myelin. They suggest that the previous observations of Branton were restricted to regions of high shadowing angle, where the particles were obscured. In untreated myelin, frozen shortly after dissection, the particle distribution was found to be uniform. If, however, the membranes were either fixed or glycerol impermeated then the particles aggregated and formed clusters in the plane of the membrane. Similar particle clustering has been observed in several other membranes, but what is remarkable about the photographs of Pinto da Silva and Miller is that the particle-rich regions in one membrane seem to line up with particle-rich regions in the adjacent membrane of the myelin sheath (this correspondence sometimes continuing across as many as 50 membranes). Furthermore, neighbouring membranes in the particle-rich regions seem more closely apposed than those in particle-free regions.

Although Pinto da Silva and Miller admit that the particle clustering itself is likely to be an artefact, they conclude that the correspondence between particle-rich regions from one membrane to the next does reveal an inter-membrane interaction which exists *in vivo*. They argue, by analogy with other freeze-fracture studies on membranes, that the particles represent protein-containing structures which traverse the lipid bilayer. They suggest that these structures stabilise the myelin sheath by interactions across the cytoplasmic and extracellular spaces. Because the fracture faces are thought to represent an essentially hydrophobic environment, Pinto da Silva and Miller argue that the protein is likely to be apolar in nature and suggest the Folch-Lees proteolipid as a possible candidate. Indeed, the fact that about 80% of the myelin protein (Folch-Lees and Wolfgram proteolipids) can be solubilised in organic solvents argues for an apolar site for these proteins. It remains to be shown, however, that the particles observed by Pinto da Silva and Miller do represent protein-containing structures which are present *in vivo*. If this is the case then current models of myelin structure may have to be revised.

Chemical antagonism in plant communities

from Peter D. Moore

ALMOST a hundred and fifty years ago De Candolle observed that the growth



A hundred years ago

M. Berthelot, the celebrated chemist, is a candidate in the moderate Republican interest for the representation in the French Chamber of Deputies, of the district in which the Institute is situated.

from *Nature*, 13, February 10, 297; 1876.

of certain weeds was injurious to crops and postulated that the immediate cause of injury could have resulted from the excretion of toxins, possibly from their roots. Back in 1925, Massey (*Phytopathology*, **15**, 773; 1925) conducted experiments which involved planting such species as potato and tomato beneath the canopy of walnut trees and observed that wilting and death of the test plants occurred wherever they were in contact with the roots of the walnut, even when they were well clear of its canopy. He also showed that portions of bark from walnut roots could cause root death in water cultured tomatoes, thus indicating the involvement of some chemical factor rather than a competitive interplay for a mineral or water resource.

The importance of such chemical interactions between higher plants, sometimes termed allelopathy, is still probably underrated in ecological circles despite some valuable recent reviews, such as those of Whittaker and Feeney (*Science*, **171**, 757; 1971) and Rice (*Allelopathy*, Academic Press, New York; 1974). This has undoubtedly resulted from the general scarcity of information relating to chemical interactions in natural communities of plants. Many potential researchers must have been discouraged by the practical difficulties involved in designing experiments to test for allelopathic interactions, especially when one considers the physicochemical complexity of the soil in which such interactions occur and also the likely involvement of soil microbial communities. It may well be that some reported allelopathic agents are in fact the products of microbial degradation of the original plant tissues rather than the direct results of a plant's metabolism. Such a possibility, however, although of academic interest, does not preclude the evolutionary development of allelopathic competitive mechanisms which involve microbial symbionts. The fact that such mechanisms favour the host plants (and thereby favour the decomposer microbe) is of prime importance to the student of plant community dynamics. This being so, it should be possible to design simple experiments which do not attempt to disentangle the peripheral question of microbial involvement, but which are concerned with the overall outcome of plant-plant interactions involving allelopathy.

Just such an experiment has recently been reported by Newman and Rovira (*J. Ecol.*, **63**, 727; 1975) who have looked for chemical interactions between a number of common British grassland species. Four grass species and four herbaceous dicotyledons were chosen, none of which had given any field indication of allelopathic mechanisms at work. Plants were grown in

buckets of sand, together with an inoculum of soil from their original habitat; it is to be expected, therefore, that the normal rhizosphere microflora was present. Leachate of these pots (and of a control pot lacking plants) was applied to all other species examined and its influence upon the growth rates and mineral content of their shoots was examined.

The leachate from certain 'donor' species had a consistent and statistically significant effect in depressing the yield of receiver species. Yorkshire fog grass (*Holcus lanatus*), catsear (*Hypochoeris radicata*) and white clover (*Trifolium repens*) were most effective in this respect. Sweet vernal grass (*Anthoxanthum odoratum*) appeared to be the most sensitive species as a receiver of leachate, while *Trifolium repens* grew faster when supplied with almost any plant leachate than under the equivalent control conditions. *Anthoxanthum* was of additional interest in that its growth when supplied with its own leachate was faster than under any other conditions. Both *Holcus*, and the crested dog's tail grass (*Cynosurus cristatus*) also showed some growth stimulation when treated with their own leachate.

Although lack of rigorous replication in this experiment precludes detailed conclusions concerning the precise interaction of individual species, it is evident from these results that allelopathic mechanisms could be of considerable importance in determining composition and pattern in neutral grassland swards. Of particular importance is the demonstration that species differ in their toxic potential and also in their sensitivity as receivers of toxins. Self stimulation amongst grassland species could be of considerable importance in the attainment of dominance within a sward, whereas self sensitivity could lead to a wider spacing of individuals and an avoidance of intraspecific competition for resources within the population. Newman and Rovira's results, in this respect, agree well with the behaviour of their species in the field, but the observations of other workers are not so simply explained. For example, McNaughton has demonstrated auto-inhibition of seed germination in the reedmace, *Typha latifolia* (*Ecology*, **49**, 367; 1968), yet this is a species which can dominate extensive areas. The selective advantage of autotoxicity may operate in different ways in different species.

By far the most important point to emerge from Newman and Rovira's data, however, is that allelopathy is a potentially important mechanism for species interactions even in situations where such chemical devices have not previously been suspected. They effectively by-pass the ecologically

irrelevant question of microbial involvement; indeed, in using only young roots in their leachate production, they could be underestimating the overall influence of allelopathy in these species if there is a microbial intermediary involved. □

Volcanic processes in ore genesis

from I. G. Gass

A packed lecture hall and an international range of contributors testified to the interest engendered by the recent joint meeting of the Institute of Mining and Metallurgy and the Volcanic Group of the Geological Society held in London on January 21–22 on the subject of Volcanic Processes in Ore Genesis. Seemingly, the timing and the topic were right and sufficient progress has been made in the area in recent years to attempt a synthesis.

Two major themes pervaded the meeting. On the one hand were the academics who, using trace element and isotope geochemistry and even naked thermodynamics, erected hypotheses and models for the tectonic setting, origin and circulation of ore-forming fluids. At the other extreme were those who provided the critical evidence as to whether these data applied to a particular ore deposit. Fortunately, speakers such as J. P. Hunt (Scripps Institution: ex-Anacosta), T. Sato (Japan Geological Survey) and G. Constantinou (Cyprus Geological Survey) with experience in both areas more than adequately bridged the all too common gulf between the two.

Stable isotope studies strongly suggest that the origin of the ore-carrying solutions was either seawater, in the case of massive sulphides formed at or near constructive margins, or meteoric waters for ore bodies such as porphyry coppers, emplaced above subduction zones. The role of magmatic waters is minimal in the former and minor in the latter; magmatic processes seem to provide the thermal energy and very little else. Both thermodynamic models of geothermal systems, as presented by J. W. Elder (University of Manchester), or modification of stable isotope ratios (E. T. C. Spooner, University of Oxford and T. H. E. Heaton, Kilbride) indicate that the circulation of the solutions that cause mineralisation follows immediately after the magmatic thermal event and that they are short-

lived in terms of thousands rather than millions of years and vigorous. There are therefore signs of a semi-quantitative breakthrough in the understanding of geothermal processes. This has been brought about by the use of a wide spectrum of analytical techniques on, for instance, the ophiolite massive sulphides of Cyprus and the porphyry copper of El Salvador. Their use elsewhere will markedly enhance our understanding of the processes involved in ore genesis.

Happily, the days when it was considered almost indecent for 'pure' academic scientists to concern themselves with 'ores' are over. A period of fruitful collaboration between hard-headed practicality and high quality academic research is, it is hoped, here to stay. □

Phyto-insanitary Britain

from a Correspondent

A LARGE proportion of the money devoted to agricultural research in the UK is spent on phytopathology and crop protection and has enabled the production of virus-free and disease-resistant crops that can be fully protected by insecticides, fungicides and herbicides, to give near maximum possible yields.

Phytopathology research within the UK has been mostly confined to known indigenous pathogens but there is an additional threat from the vast amounts of plant material imported in many different forms every day. This comes from all over the world and frequently carries pathogenic organisms or strains of pathogens alien to this country. Fortunately most of these pathogens cannot survive in the unfavourable climate but the few that can may cause economically serious diseases.

Many such diseases have already entered the UK through the importation of infected plant material. Fire-blight of apples and pears, for example, was unheard of in Britain 20 years ago, though it was widespread in North America. It is now an established disease in southern England affecting hawthorns, many ornamental species of Rosaceae and posing a potential threat to apple and pear species, though disease outbreaks have been mostly confined to South-East England (*Ann. appl. Biol.*, **69**, 137; 1971). This disease has put severe restrictions on nurserymen who wish to export apple and pear varieties. Tobacco ringspot virus has also been introduced recently on pelargoniums imported from the USA.

The fruit industry suffered another setback recently when the Ministry of Agriculture reported that at least 150 acres of plums have some levels of infection with plum pox (sharka) virus, an aphid-borne virus. This potentially devastating disease originated in south-eastern Europe but has now spread throughout most of the continent. The Germans unknowingly introduced plum pox into their own plums from propagating material brought back from Yugoslavia after the last war, which resulted in serious losses in crop production in some regions. The virus is now endemic in many parts of Eastern Europe infecting a wide range of *Prunus* species (*Acta. Horticulturae*, **44**, 155; 1975).

Britain, through the Nuclear Stock Association (Tree Fruits), has been producing virus-free plums for nurserymen and fruit farmers for several years. Ironically a wide range of *Prunus* species from all over Europe is also imported even though the dangers of plum pox have been apparent for many years (*Pl. Pathol.*, **17**, 66; 1968). Much of this material is accompanied by phytosanitary certificates of the exporting countries which are not necessarily the countries of origin. These certificates state that the material has been inspected and is certified free of certain diseases.

Most virus diseases cannot be detected by visual inspection, however, particularly in dormant plants in transit. When the material arrives in the UK it is again inspected by the Ministry of Agriculture Plant Health Inspectorate who are not equipped to detect most virus infections at this stage. The plants are then distributed to nurseries for propagation, and are often grown close to virus-free rootstocks and mother-trees in the nuclear stock scheme. In the retesting of nuclear stocks carried out last year some plum pox infection was found.

As plum pox severely affects Victoria plum, the most widely grown cultivar in the UK, the survival of the plum industry is threatened unless steps are taken to eradicate this disease.

Because of the present loopholes in the system, which have been apparent for many years, particularly for virus diseases, it may only be a matter of time before diseases such as apple proliferation, oak wilt and pear moria are introduced.

Unlike countries such as New Zealand and the USA, the UK is failing to implement effective phytosanitary measures. Farmers and growers in Britain are not getting the protection they require and production is being restricted or made more costly by the introduction of new diseases. For too long exporting countries have been able to sell sub-

standard and infected plant material, undercutting local industries and threatening the health of home-produced material. It is time that the Ministry of Agriculture and Plant Health Branch heeded the warnings of research workers and implemented effective phytosanitary procedures. □

Observing satellites

from a Correspondent

On January 17, 1976, there was an all-day meeting, at the Royal Society, in London, of optical observers of satellites and the university researchers who are, or soon will be, using their observations. The meeting was organised by the Optical Tracking Subcommittee of the British National Committee on Space Research, and the stimulus was the recent award by the Science Research Council to the Universities of Aston and Leicester of grants for research projects based on optical observations of artificial satellites. The current observing effort is to be augmented by recruiting a second team for the Hewitt Camera at Malvern and an increase in staff in the Satellite Orbits Group at the Appleton Laboratory, Slough, which provides the prediction service.

THE morning session began with an outline of the university proposals from their initiators, C. J. Brookes (University of Aston) and A. J. Meadows (University of Leicester). Brookes's programme includes a varied group of geophysical studies based on orbital analysis. The aims are to obtain better values of zonal harmonics in the geopotential, to study the variation of atmospheric rotation rate and air density during a solar cycle, and to study in detail the radiation pressure effects on orbits, using balloon satellites. Meadows proposed to concentrate on two topics: first, analysis of resonant lunisolar perturbations, by intensive observation of satellites at particular inclinations (56.06° being a promising starter); and second, studies of spin rates of similar satellites in orbits at different heights, to distinguish the effects of atmospheric and magnetic damping in the hope of measuring the variations in both.

After these plans for the future, some recent results from orbital analysis were presented. D. M. Brierly (Royal Radar Establishment, Malvern) described his determination of air density at a height of 128 km from a

THERE have been many studies of nucleon transfer reactions at low energies (5 to 50 MeV) and these have given much information on the single particle states of nuclei (see for example, *Nature*, **254**, 18; 1975; **256**, 615; 1975). Very few analyses have been made at higher energies, partly because it is then difficult to resolve the final states and partly because it is thought unlikely that such studies could give any information that could not be obtained from the technically much easier studies at lower energies.

In 1974 a group working at the Saturne synchrotron (*Phys. Lett.*, **52B**, 57) published the results of their measurements of the $^{12}\text{C}(\text{p},\text{d})^{11}\text{C}$ neutron pickup reaction at 700 MeV. These data were remarkable because the energy resolution was sufficient to resolve several states of ^{11}C , and thus provided the only stripping or pickup data available above 200 MeV.

The group made a preliminary analysis of their data using the distorted wave formalism, with the zero-range approximation for the neutron-proton force. At high energies, however, the momentum transfers are large, and this invalidates the approximation. They also ignored the D-state component of the deuteron wavefunction, although this is expected to contribute strongly at high energies. In spite of this, they found that the overall shape of the differential cross section is given quite well by their calculation, as shown in the figure, but the absolute magnitude is wrong by a factor of two.

In the past few years computer programs using a finite range neutron-proton force have been developed,

mainly to analyse heavy ion reactions. Rost and Shepard (*Phys. Lett.*, **59B**, 413; 1975) have now used one of these programs to analyse the $^{12}\text{C}(\text{p},\text{d})^{11}\text{C}$ data, and have also included the effect of the D-state of the deuteron.

As shown in the figure, they found that the theory then gives a cross-section in good agreement with the experimental data, both in absolute magnitude and in overall shape. As expected the contribution of the D-state is dominant, so the previous agreement in shape must be largely fortuitous. It is thus very important

Neutron pickup at high energies

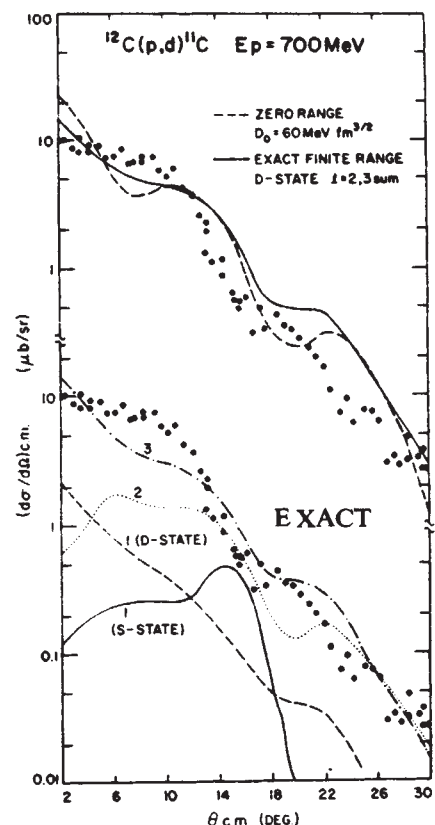
from P. E. Hodgson

for reactions of deuterons at high energies to use an accurate deuteron wavefunction, and the results can themselves provide information about that wavefunction.

This conclusion is reinforced by studies of the contribution of the S-state component of the deuteron wavefunction. Rost and Shepard used an S-state waveform calculated from the Reid soft-core potential, that has been obtained by fitting a large body of nucleon-nucleon and deuteron data. Calculations with the Hulthén wavefunction, a simple analytical function widely used at low energies, gave cross sections completely in disagreement with the experimental data.

This work shows the value of repeating familiar analyses at sub-

stantially higher energies, whenever it is practicable to do so.



Differential cross section for the $^{12}\text{C}(\text{p},\text{d})^{11}\text{C}$ reaction at 700 MeV compared in the top section with zero-range and exact finite-range distorted wave calculations. The zero-range curve has been arbitrarily divided by two to fit the data. The lower section shows the individual cross sections for the S-state $L=1$ and the D-state $L=1,2,3$ contributions.

decaying Molniya satellite, entirely on the basis of visual observations from Britain. Brookes presented the results of his first orbit determination, of Cosmos 373 (1970-87A) at 25 epochs between 1971 and 1975: this orbit, at inclination 62.9° , is closer to the critical inclination of 63.4° than any other orbit that has been accurately analysed, and he has determined the oscillation in perigee height caused by odd zonal harmonics in the geopotential as 59 km—the largest value yet established. D. G. King-Hele (Royal Aircraft Establishment, Farnborough) described new results obtained by himself and D. M. C. Walker in determining the atmospheric rotation rate from orbital analysis; the variations with height and local time have now been separated, and three curves of the variation of rotation rate with height were shown, for average local time, for evening (18-24 h) and morning (4-12 h).

The afternoon session began with an

excellent presentation by J. Eady (Ordnance Survey) of the work of the Hewitt Camera at Malvern, owned and operated by the Ordnance Survey. He commended the beautiful design of this accurate camera, a great tribute to the skill of the late Mr Hewitt, and looked forward to its increased use with a second observing team. The session continued with a succession of short contributions on topics connected with satellite observing. D. A. Richards (University College of Wales, Aberystwyth), offered some thoughtful comments on the slower time response of the eye in poor light, and demonstrated how the eyes see the linear swing of a pendulum as an ellipse if the light to one eye is reduced by a filter. The other contributions, to flash through them even more rapidly than they were presented included a kaleidoscope of satellite shapes (J. A. Pilkington); an experimental demonstration of flash behaviour with satellite models (M. D.

Waterman); recent work on radio tracking of satellites at Kettering and associated stations (G. E. Perry); the telemetry of Soviet navigation satellites (C. D. Wood); a possible explanation of the break-up of the Pageos balloon and several other US satellites (R. D. Eberst); the US Navy satellite tracking network (D. G. King-Hele); an analysis of a lengthy questionnaire on observing previously completed by the participants (M. D. Waterman); and a film of the Apollo-Soyuz Mission.

Apart from the formal presentations the meeting was intended to allow the visual observers to meet the new university researchers; to introduce the latter to some of the problems of observing; and to allow the volunteer visual observers to meet each other, to renew their enthusiasm, and to see how their unpaid work was utilised by the scientists. The subsequent comments of the participants suggested that these aims were fully achieved. □

articles

Head-tail radio sources in the cluster of galaxies Abell 1314

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Sterrewacht, Leiden, The Netherlands

A high resolution study of the cluster of galaxies Abell 1314 has revealed two 'head-tail' radio sources, associated with the galaxies IC708 and IC711. The tail of IC711 extends fully 820 kpc. The source properties are described and discussed in terms of the 'radio trail' model.

THE 'head-tail' radio sources are characterised by a highly elongated radio structure, near one end of which lies the associated optical galaxy—usually a giant elliptical. The first examples of these tadpole shaped objects were discovered by Ryle and Windram¹ in the Perseus cluster, and roughly a dozen have now been recognised, mainly through observations with the aperture synthesis instruments at Cambridge and Westerbork. Most, perhaps all, of these sources are associated with clusters of galaxies. A compendium of currently recognised 'head-tail' galaxies is presented in Table 1, along with their more salient properties.

The most satisfactory explanation of these objects is the 'radio trail' hypothesis². As the galaxy ploughs through a dense intracluster gas at supersonic speed, it is envisaged to eject pairs of plasmoids in opposite directions, as commonly assumed for 'normal' double radio galaxies. These blobs then brake by interaction with the intracluster medium, and form a trail behind the galaxy. Jaffe and Perola³ developed two models based on this idea. In the first (the 'independent blob' model), the dynamic and radiative evolution of a series of ram pressure confined plasmoids was calculated. This model has difficulties with the spectral data and so they considered a second case (the 'magnetospheric' model), in which the galaxy is endowed with a strong magnetic field, drawn out into a magnetotail by the rapid motion through the surrounding gas. The double structure and symmetry of the tails^{2,4-7}, and the detection of compact sources at the positions of the galaxies themselves^{5,7}, speak strongly in favour of the 'radio trail' hypothesis; and the polarisation data on NGC1265 (ref. 9) indicate the magnetic field to be aligned more or less along the tail, as might be expected in models invoking galaxy motion through an intracluster gas (for example the 'magnetospheric' model).

It is important to discover more of these objects to attack the following problems: first do 'head-tail' galaxies occur preferentially in rich clusters? Much needed information on the elusive intergalactic medium could thus be obtained. Second, can the shape of the tails be completely accounted for in terms of the orbit of the galaxy in the cluster's gravitational field, or are additional processes (for example 'gales', or a buoyancy effect in the intracluster gas) required to explain the pronounced curvature observed in some sources? Third, is there any

tendency for these objects to occur in clusters which also contain other active radio galaxies (see ref. 4)? Fourth, are the energy losses of the relativistic electrons by synchrotron radiation, inverse Compton scattering, and adiabatic expansion so severe that particle acceleration is required a large distance from the parent galaxy?

We report here two new 'head-tail' galaxies (IC708 and IC711), both located in the cluster of galaxies Abell 1314, and discuss their structure and interrelationship. This work forms part of a continuing programme to search for such sources with the Westerbork Synthesis Radio Telescope (WSRT).

Selection of objects and observations

Owen^{10,11} has surveyed over 500 Abell clusters of galaxies with a resolution of 10' at $\lambda = 21$ cm, and presented contour maps of sources with complex or multiple features. Although his map of the radio emission from Abell 1314 shows extended structure, the resolution is inadequate to delineate the nature of the source and determine unambiguously with which cluster galaxy, if any, it is associated. This field seemed a promising candidate for higher resolution investigation, so we observed it with the WSRT at $\lambda = 49$ cm (610 MHz) and $\lambda = 6$ cm (4,995 MHz). The former wavelength, where the resolution is $\sim 1'$, is ideal for the detection of faint, extended features, whereas the latter enables a higher resolution ($\sim 6''$) investigation of the more intense, compact regions.

The WSRT and the procedure for data analysis have been fully described elsewhere^{12,13}. Briefly, the instrument consists of twelve 25-m parabolic antennae along an east-west line. Ten of these (telescopes 0 to 9) are fixed and the other two (telescopes A and B) are moveable along a rail track. The signal from each of the ten fixed telescopes is correlated with that from each of the two moveable ones, resulting in twenty simultaneous baselines ranging from the separation 9-A to 9-B + (19 × 72 m) at intervals of 72 m. Fourier transformation and linear combination of the correlated signals yield maps of the four Stokes parameters¹⁴. The reception pattern of the individual paraboloids causes emission away from the field centre to be attenuated. For the extended radio structure associated with IC711 and IC708, the attenuation at $\lambda = 49$ cm is always < 3%. At $\lambda = 6$ cm, the maximum attenuation of the extended structure on the maps of IC711 and IC708 is 15% and 6% respectively. Both the contour maps and the quoted flux densities have been corrected for these small effects. The observational parameters, sensitivities and highest resolution obtainable are summarised in Table 2.

Results at 49 cm and optical identifications

Figure 1a and b is a radio photograph and a contour plot of the brightness (total intensity) distribution in the central region of Abell 1314 at $\lambda = 49$ cm (the cluster diameter is

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Table 1 Confirmed head-tail galaxies

Optical galaxy position (1950.0)	Name of head-tail galaxy	Name of cluster of galaxies	Cluster redshift (z)	Optical magnitude (m)	Flux density at $\lambda = 21$ cm (Jy)	Power at $\lambda = 21$ cm (10^{24} W Hz $^{-1}$)	Angular size of galaxy tail (arc min)	Corresponding linear size (kpc)	First shown 'head-tail' by (reference)
02 h 55 m 47 s +13° 22'	4C13.17A	Abell 0401	(0.08)	—	0.38	10.0	6.5	780	20
03 13 25 +41 08	IC310	Abell 0426	0.0183	14	0.80	1.0	8.5	260	1
03 14 06 +41 16	C. & R. 15	Abell 0426	0.0183	15	0.03	0.04	2.5	80	2
03 14 56 +41 40	NGC1265	Abell 0426	0.0183	14	7.70	10.0	10.5	320	1
04 45 32 +44 55	3C129	Unnamed	0.0213	17	9.00	20.0	14.0	500	4
11 31 15 +49 20	IC708	Abell 1314	0.0335	14	(0.75)	(4.0)	1.5	80	this paper
11 32 03 +49 14	IC711	Abell 1314	0.0335	14	(0.55)	(3.0)	15.0	820	this paper
11 56 59 +28 11	NGC4869	Abell 1656	0.0230	14	0.45	1.0	4.5	170	21
12 15 49 +35 08	NGC6109	Zw1615+3505	0.0296	15	3.00	10.0	12.0	590	22
16 21 17 +38 02	NGC6137	Unnamed	0.0310	14	0.43	2.0	1.0	50	22
16 58 19 +32 39	4C32.52E	Abell 2241	(0.066)	16	0.20	4.0	1.5	150	23
17 12 05 +64 05	4CT64.20.1	Abell 2255	(0.07)	16	0.26	5.0	1.5	160	20
22 47 25 +11 21	NGC7385	Zw2247+1107	0.0268	13	2.30	7.0	12.0	530	8

Redshift values in parentheses are estimated from apparent magnitudes. Flux densities and powers at $\lambda = 21$ cm in parentheses are estimated from scaling down the corresponding values at $\lambda = 49$ cm using a spectral index of -0.7 . A Hubble constant $H_0 = 50$ km s $^{-1}$ Mpc $^{-1}$ and a deceleration parameter $q_0 = 1$ have been used throughout to compute the powers at $\lambda = 21$ cm and the corresponding linear sizes.

$\sim 2^\circ$). A highly elongated radio structure can be traced on a scale of $15'$, at the southern end of which, but still within the radio contours, lies the galaxy IC711. We identify the elongated feature as a 'head-tail' source associated with this galaxy, for the relative position of optical and radio features is typical of such objects and our observations at $\lambda = 6$ cm (see next section and Fig. 2) show IC711 to be active. If the extended structure to the north of declination $49^\circ 22'$ and elongated in position angle $\sim 30^\circ$ is, in fact, a part of the tail and if IC711 is a member of the cluster, the overall length of the source is > 800 kpc ($H_0 = 50$ km s $^{-1}$ Mpc $^{-1}$). IC711 is then the longest 'head-tail' galaxy known, with a distance ~ 200 Mpc. The tail of IC711 passes to the east of another well resolved, but more intense and compact, source at declination $49^\circ 20'$. This source is identified with the galaxy IC708, although the apparent peak of the emission at $\lambda = 49$ cm is displaced some $20''$ from the optical centroid. Observations of IC708 at $\lambda = 6$ cm (Fig. 3) show, however, that it is both active and possesses a well developed radio trail, fully accounting for the displacement apparent in the lower resolution map at $\lambda = 49$ cm. Webber¹⁵ observed Abell 1314 at three radio frequencies and detected two sources which he identified with IC709 and IC711. Our observations show the former identification to be incorrect. Although the galaxy IC709 does lie close to the tail of IC711 (between IC711 and IC708), we do not detect it, allowing an upper limit for its flux ($S_{49\text{ cm}} < 10$ mJy) to be established.

In the central part of the cluster, two other bright galaxies are detected at $\lambda = 49$ cm. IC712, an elliptical galaxy with $m_v \simeq 14$ (A. Oemler, personal communication), lies $\sim 7'$

to the north of IC711, and coincides with an unresolved radio source (RA (1950.0) = 11 h 32 min 06.47 s, dec. (1950.0) = $+49^\circ 21' 15.6''$) with flux density $S_{49\text{ cm}} = 58$ mJy. The

Fig. 1 The brightness (total intensity) distribution in the central part of the cluster of galaxies Abell 1314 at $\lambda = 49$ cm and resolution $52'' \times 69''$. *a*, Radio photograph (kindly made by W. Jaffe). *b*, Contour map. In *b*, the crosses show the positions of the optical galaxies, measured by Oemler (personal communication), and the half-power beam width is represented by the ellipse in the bottom right hand corner; the contour values are: 5, 10, 15, 25, 50, 100, 150, 200, 350 and 500 mJy (beam area) $^{-1}$.

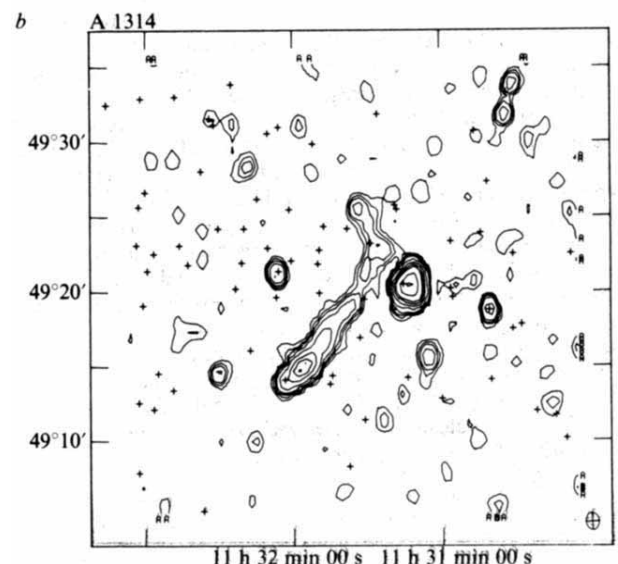
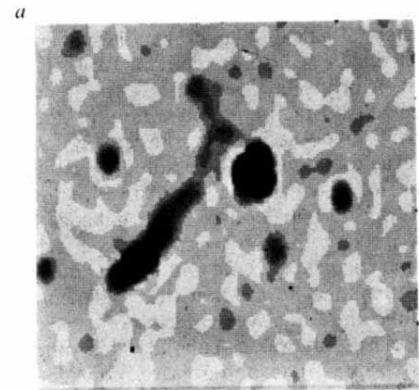


Table 2 Observational parameters

Galaxy observed	Whole cluster	IC708	IC711
Field centre RA (1950.0)	11 h 32 min 00 s	11 h 31 min 12 s	11 h 32 min 00 s
dec. (1950.0)	$49^\circ 20' 00''$	$49^\circ 20' 00''$	$49^\circ 14' 00''$
Wavelength (cm)	49	6	6
Observing time (h)	1×12	1×12	1×12
Minimum baseline (m)	72	36	54
Spacing increment (m)	72	72	72
Observing period	August 1974	April 1975	March 1975
Highest resolution, RA $\times$ dec. (arc s $\times$ arc s)	52×69	6×8	6×8
HPBW of individual paraboloids (arc min)	82	10	10
Radius of first grating response, RA $\times$ dec. (arc min $\times$ arc min)	24×32	3×4	3×4
R.m.s. noise (mJy (beam area) $^{-1}$)	1.6	1.2	1.2

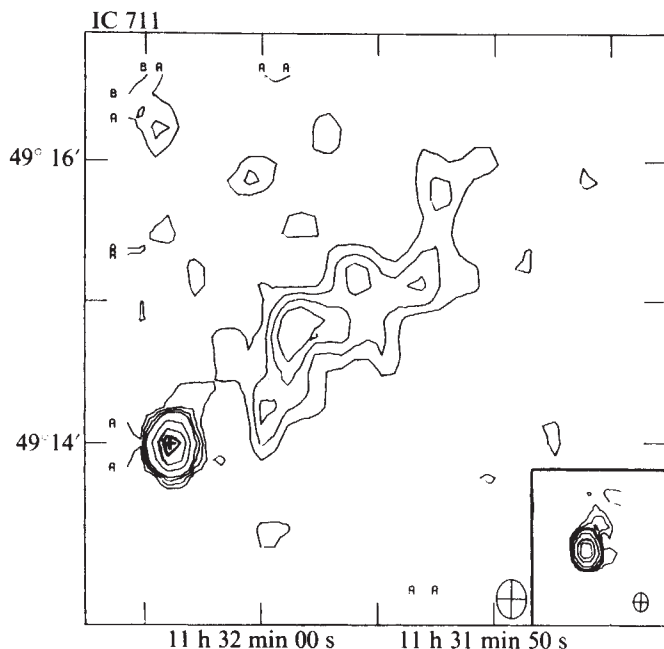


Fig. 2 The brightness (total intensity) distribution in the galaxy IC711 at $\lambda = 6$ cm. The half-power beam width ($12'' \times 16''$) is shown by the ellipse in the bottom right hand corner and the cross marks the centroid of the optical galaxy. The contour values are: 2.5, 5, 7.5, 10, 20, 30 and 40 mJy (beam area) $^{-1}$. The inset in the bottom right hand corner shows the $\lambda = 6$ cm nuclear source at a resolution of $6'' \times 8''$, with contour values: 1.25, 2.5, 3.75, 5, 10, 20 and 30 mJy (beam area) $^{-1}$.

second galaxy ($m_v = 15.7$) is a spiral with a flux $S_{49\text{cm}} = 70$ mJy and radio position RA = 11 h 30 min 40.45 s, dec. = $+49^\circ 18' 49.8''$. If this last galaxy is a member of Abell 1314, its radio power at $\lambda = 49$ cm is 3×10^{23} W Hz $^{-1}$; it is unresolved at $\lambda = 49$ cm. Markarian 178, an object possessing a strong ultraviolet excess, lies $\sim 14'$ north-west of IC712, and is not detected in our survey, implying that its flux $S_{49\text{cm}} < 5$ mJy; its redshift ($v_r = 270$ km s $^{-1}$) is much lower than that of the cluster, and Sargent¹⁶ has suggested that it is a dwarf emission-line galaxy. A more complete discussion of the optical identifications of other radio sources in the field will be presented elsewhere.

Results at 6 cm

To explore the structure of the brighter parts of IC711 and IC708 with finer resolution, we have observed them with the WSRT at $\lambda = 6$ cm. The map of IC711 with resolution $12'' \times 16''$ (Fig. 2) shows an unresolved source, whose position agrees well with the centroid of the optical galaxy (as measured from the blue Palomar Sky Survey print) and is presumably to be identified with its nucleus. To the north-west of this source lies the tail, the brighter parts of which are detected up to $\sim 3'$ from the galaxy. Between the brightest part of the tail and the nucleus, little radio emission is found. On the 'radio trail' hypothesis, this would imply that IC711 has not ejected double plasmoids recently. A close examination of the radio contours within $10''$ of the compact source at a resolution of $6'' \times 8''$ (see inset in Fig. 2) yields, however, evidence for a double feature apparently emerging from the nucleus. Although the brightness of this feature is only about twice r.m.s. noise, it is interesting that it is 'swept back' in the general direction of the tail itself. Basic data relevant to IC711 (and IC708) are summarised in Table 3.

The structure of IC708 at $\lambda = 6$ cm is shown in Fig. 3. The intense resolved source on the $\lambda = 49$ cm map (Fig. 1) superimposed on IC708 may now be seen to consist of four components. The brightest, unresolved at $\lambda = 6$ cm, lies close

to the centroid of the optical galaxy (Table 3) and is, as in the case of IC711, presumably to be identified with the nucleus. Stretching $> 1'$ to the west of the nucleus are two well resolved strands, implying that IC708 is also a 'head-tail' galaxy. There is no reason to believe that the fourth component, $\sim 1'$ of the nucleus, is physically related to IC708. The nuclear source is weakly (three times r.m.s. noise) linearly polarised (see Table 3).

Discussion

Our observations of IC708 and IC711 are consistent with the 'radio trail' model in its general form². The observations at $\lambda = 6$ cm show the two galaxies to be active, implying that the nucleus is the supplier of energy in both cases, as is known to occur in 'normal' radio galaxies. The source morphologies strongly suggest a radio trail, particularly for IC708. A more careful scrutiny of the properties of IC711, however, reveals a difficulty with the simple models developed by Jaffe and Perola³. This problem centres around the large size of the source, an aspect we now discuss.

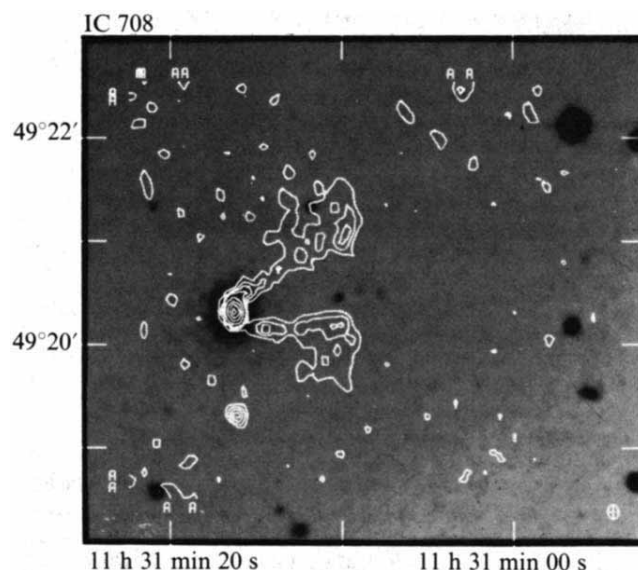


Fig. 3 A contour map of the brightness (total intensity) distribution in IC708 at $\lambda = 6$ cm and resolution $6'' \times 8''$, superimposed on the blue Palomar Sky Survey print. The half-power beam width is shown by the ellipse in the bottom right hand corner, and contour values are: 2.5, 5, 7.5, 10, 25, 50, 75 and 100 mJy (beam area) $^{-1}$.

In both the 'independent blob' model and the 'magnetospheric' model, the relativistic electrons are envisaged to be accelerated within the optical galaxy rather than in the tail itself. We now show that severe difficulties result from this assumption in the case of IC711. An electron radiating at $\lambda = 49$ cm in a typical equipartition magnetic field in the tail of 4×10^{-6} gauss has a half life to synchrotron radiation and to inverse Compton losses on the microwave background of $t_{1/2} = 9 \times 10^7$ yr. Writing L = length of tail = 820 kpc, the average velocity of propagation V of such electrons with respect to the galaxy must be $\geq L/t_{1/2} = 8,600$ km s $^{-1}$, if they are accelerated within the galaxy. In the 'independent blob' model, the maximum possible V is simply the velocity of the galaxy with respect to the intracluster gas V_g (since ram pressure eventually brings the plasmoids to rest), which, if the galaxy is a member of the cluster, is surely much smaller than the above value. The value of V appropriate for the 'magnetospheric' model is less obvious since, although it is to be expected that the relativistic particles propagate through the thermal gas at about the Alfvén speed V_a (refs 17 and 18), the velocity of the thermal plasma in the

tail V_t with respect to the galaxy is unknown. Jaffe and Perola took $V_t = V_g$, so the maximum possible is $V = V_g + V_a$. The assumption $V_g \leq 8,600 \text{ km s}^{-1}$ then implies $V_a \geq 8,600 \text{ km s}^{-1}$, that is, a thermal particle density $\rho \leq 2 \times 10^{-30} \text{ g cm}^{-3}$. Implicit in the 'magnetospheric' model is a rather uniform magnetic field aligned along the tail, and, with such a low gas density, appreciable polarisation could be expected at $\lambda = 49$

duration of past activity in IC711 itself, presuming the intra-cluster gas to have a distribution similar to that of the galaxies. The much shorter length of the tail of IC708 could be accounted for if it has just entered the western edge of the dense gas. The apparent expansion of the two strands of IC708 near RA = 11 h 31 m 11 s (see Fig. 3) is indeed suggestive of a reduced gas density there.

Table 3 Some data on IC711 and IC708

Source parameter	IC711	IC708
Positions (1950.0): radio nucleus	RA = 11 h 32 m 03.90 $\pm$ 0.05 s dec. = 49° 13' 57.8 $\pm$ 0.6''	RA = 11 h 31 m 16.27 $\pm$ 0.05 s dec. = 49° 20' 19.3 $\pm$ 0.6''
centroid of optical galaxy*	RA = 11 h 32 m 03.95 $\pm$ 0.1 s dec. = 49° 13' 56.5 $\pm$ 1.0''	RA = 11 h 31 m 16.36 $\pm$ 0.1 s dec. = 49° 20' 18.1 $\pm$ 1.0''
Visual magnitude	14.8	14.4
Angular size of galaxy-tail at $\lambda = 49 \text{ cm}$ (arc min)	15	1.5
Corresponding linear size in plane of sky at $\lambda = 49 \text{ cm}$ (kpc)†	820	80
Linear size of nucleus (FWHM) at $\lambda = 6 \text{ cm}$ (kpc)†	< 3	< 3
Flux densities (mJy): total, $\lambda = 49 \text{ cm}$	1,047 $\pm$ 100	1,432 $\pm$ 70§
nucleus, $\lambda = 6 \text{ cm}$	44 $\pm$ 3	114 $\pm$ 5
Linear polarisation of nucleus: percentage (%) at $\lambda = 6 \text{ cm}$	< 6	3.6 $\pm$ 1.2 (r.m.s.)
position angle (°) at $\lambda = 6 \text{ cm}$	—	134 $\pm$ 10
Power at $\lambda = 49 \text{ cm}$ (W Hz ⁻¹)†	5 $\times$ 10 ²⁴	7 $\times$ 10 ²⁴
Radio luminosity (W)†	5 $\times$ 10 ³⁴	8 $\times$ 10 ³⁴
Minimum energy in tail (erg)‡	5 $\times$ 10 ⁵⁸	1 $\times$ 10 ⁵⁸
Equipartition magnetic field in tail (gauss)‡	(2–6) $\times$ 10 ⁻⁶	7 $\times$ 10 ⁻⁶

*Measured from the blue Palomar Sky Survey print with the 'Coradigraph' measuring machine at the Royal Greenwich Observatory.

†Assuming $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, deceleration parameter $q_0 = 1$, and a radio window extending from 10 MHz to 100,000 MHz.

‡Minimum energy estimates have been made assuming the total energy in cosmic ray protons to be equal to that of the electrons.

§Preliminary results at $\lambda = 21 \text{ cm}$, when combined with those at $\lambda = 6 \text{ cm}$, indicate that the background source I' to the south of the nucleus of IC708 (see Fig. 3) has a spectral index $\alpha = -0.6 \pm 0.1$. Extrapolation of this spectrum to $\lambda = 49 \text{ cm}$ yields $S_{49 \text{ cm}} = 55 \text{ mJy}$. The flux quoted for IC708 represents the flux of the whole source on the $\lambda = 49 \text{ cm}$ map minus the above flux of the background source.

cm, since the Faraday rotation would be very low. Our data, however, show the tail of IC711 to be < 3% linearly polarised at $\lambda = 49 \text{ cm}$ in the brighter parts. Of course, a more random magnetic field could account for the lack of polarisation, but in that case relativistic electrons should diffuse down the tail more slowly. These arguments suggest that the relativistic electrons radiating at $\lambda = 49 \text{ cm}$ can probably not propagate from the nucleus to the end of the tail in the face of the energy losses. This conclusion is true *a fortiori* if the tail does not lie in the plane of the sky, if the magnetic field is stronger or weaker than the assumed equipartition value, or if adiabatic losses are also included. Our discussion implies, therefore, that the relativistic particles may be reaccelerated within the tail of IC711 itself, or somehow replenished from the intracluster medium. The latter possibility has been proposed by W. A. Christiansen (personal communication).

The size of IC711 and IC708 visible on the Palomar Sky Survey red print are at least 25'' and 65'' respectively. Examination of Figs 2 and 3 shows that the radio-emitting plasmoids are already swept back when < 10'' from the nuclei, well within the optical extents. A similar conclusion may be drawn from the $\lambda = 6 \text{ cm}$ map of NGC1265 (ref. 7), and constitutes rather direct evidence that the intracluster gas blows through the galaxies, probably sweeping out any pre-existing gas, as has been proposed on theoretical grounds¹⁹. This conclusion depends only on the validity of the 'radio trail' hypothesis in its most general form.

The size (FWHM) of the distribution of optical galaxies in Abell 1314 is, after correction for the background galaxies, ~ 20' and 15' in RA and dec. respectively. The latter value is close to the length of the tail of IC711, and we speculate that the extent of its radio trail is determined by the scale of the intra-cluster gas necessary to contain the plasmoids, rather than the

It is intriguing to note the change in position angle of elongation in the tail of IC711 at dec. = 49° 22', quite near IC708. There is, however, no definite evidence for any interaction between the two sources, and the peculiar morphology of IC711 could be accounted for in terms of projection effects, an elliptical orbit through the cluster being viewed obliquely.

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T4 gene 32 protein model for control of activity at replication fork

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Limited hydrolysis of gene 32 protein by various proteinases results in the production of three stable cleavage products. Two of these products show an affinity for native T4 DNA cellulose that the uncleaved protein does not exhibit. A model for proteolytic cleavage and for the local unwinding of DNA in advance of the replication fork is discussed in terms of this unusual binding affinity.

GENE 32 protein (P32) of bacteriophage T4, isolated and characterised by Alberts *et al.*¹, is essential for DNA replication² and recombination^{3,4}. It has the unique ability to convert duplex DNA to single strands at a temperature that is 40 °C below the melting temperature (T_m) of the DNA and is therefore thought to be responsible for local unwinding of duplex DNA in advance of the replicating fork. This ability to denature duplex DNA results from strong cooperative binding of P32 to single strands with little or no binding to double-stranded regions. Preferential binding of P32 to single strands, besides melting the duplex, has the reverse effect of facilitating base-pair alignment between single strands. Presented with single-stranded DNA, P32 induces a chain conformation (in the DNA) which increases the reannealing rate of homologous strands, a reaction which might be important to recombination.

Direction of the reaction denaturation $\leftrightarrow$ renaturation in the presence of P32 depends on stability of the duplex. At levels of ionic strength and Mg^{2+} concentration presumed to be present *in vivo*, the T_m of T4 DNA would be about 85 °C and at physiological temperatures the presence of P32 alone would be inadequate to keep the duplex apart in advance of the moving replication fork.

A protein of molecular weight 27,000, which copurified with P32 (35,000) has been shown to denature T4 DNA and bind cooperatively to the denatured DNA. In the same conditions P32 could do neither⁵. This protein was named P32* because it was derived from P32. Here, we show that production of P32* is the result of a protease contaminant introduced during purification and that treatment of P32 with chymotrypsin, trypsin or pepsin, each produces P32*-like molecules (27,000) and two other partial hydrolysates (34,000 and 26,000). We tested the binding of these limited hydrolysis products to single- and double-stranded DNA and found different combinations of affinities for each. A possible relationship between proteolysis and the binding/denaturing abilities of these products is discussed in terms of a model of reversible denaturation of duplex DNA.

Origin of proteolytic activity in purified P32 preparations

On storage of purified P32 at 4 °C, a second component of lighter molecular weight (27,000) increased at the expense of P32 (ref. 5). This suggested that a protease

contaminant had copurified with P32 and was slowly producing P32* from P32 by limited hydrolysis.

Three possible sources of proteolytic contamination were examined: a protease from *Escherichia coli*, a T4-induced protease, or contamination of pancreatic DNase I used during the preparation of cell extracts from which P32 was purified. T4 is known to induce a proteolytic activity related to processing of its head precursor proteins. This activity is present in lysates from some head negative (gene 23) mutant-infected cells and is absent in gene 21 mutant-infected cells⁶.

Purified ³⁵S-labelled P32 was treated with cell extracts from: *E. coli* cells infected with a gene 23 mutant (*amb17*); *E. coli* cells infected with a gene 21 mutant (*amN90*); uninfected *E. coli* cells; or a solution of pancreatic DNase I. Results are shown in Fig. 1; only the DNase solution produced any appreciable amount of proteolysis as judged by a decrease in molecular weight after 1 h incubation at 37 °C.

Commercially available DNase is known to be contaminated with chymotrypsinogen B (ref. 7). Purification of the DNase to remove chymotrypsinogen B (ref. 7) before preparation of cell extracts resulted in isolation of P32 with a greatly reduced amount of copurifying P32*. Incubation of purified ³⁵S-labelled P32 with DNase pretreated with tosyl-phenylalanyl-chloromethyl-ketone (TPCK) caused substantial reduction in the amount of hydrolysis products formed. Therefore it seems likely that hydrolysis of a trace amount of chymotrypsinogen B to chymotrypsin B was mainly responsible for the P32* found in aged P32 preparations.

Limited hydrolysis of P32 with known proteinases

Having established that production of P32* resulted from protease contamination, we decided to try and produce P32* from P32 by proteolysis with known proteinases. If this could be done then we could design and undertake experiments to determine why P32* is able to denature T4 DNA and P32 cannot.

Treatment of P32 with three proteinases, bovine- α -chymotrypsin, bovine trypsin, and bovine pepsin in varying conditions (substrate concentration 0.2 or 0.86 mg ml⁻¹; enzyme concentration 0.1–100 μ g ml⁻¹; temperature 4 or 37 °C; reaction time 1 h at pH 8.1) produced three products: P32*-I, -II and -III with molecular weights of 27,000, 34,000 and 26,000 respectively (for mechanism, see discussion under model for proteolysis). Conditions needed to reach a specific stage of hydrolysis differed with enzyme used but there was a definite order of appearance and disappearance of the three products. This order together with the position of each product after electrophoresis in SDS gels (12.5% acrylamide) is presented in Fig. 2.

In general, in the mildest conditions, P32*-I, a molecule with the same mobility as that of P32* appeared. With increasing enzyme concentrations two new products,

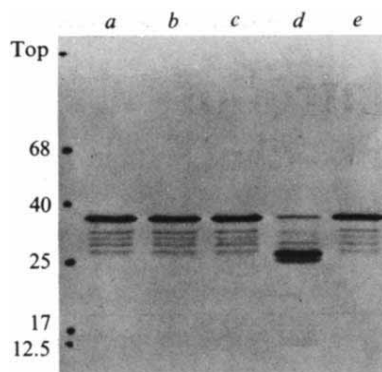


Fig. 1 Autoradiogram of ^{35}S -labelled gene 32 protein analysed on SDS gels. P32 treated with: *a*, T4 *amB17* (gene 23)-infected *E. coli* cell extract; *b*, *amN90* (gene 21)-infected cell extract; *c*, uninfected cell extract; *d*, $100\ \mu\text{g ml}^{-1}$ pancreatic DNase I (Worthington); *e*, untreated control. Markings with corresponding numbers along left-hand side denote position and molecular weight ($\times 10^3$) of reference proteins marked with radioactive ink before autoradiograms were taken. 68, bovine serum albumin; 40, ovalbumin; 25, chymotrypsinogen; 17, myoglobin; and 12.5, cytochrome *c*. Treatment of ^{35}S -labelled P32 with cell extracts: ^{35}S -P32 was added to the cell extracts to give a final concentration of $200\ \mu\text{g } 2.5 \times 10^5\ \text{c.p.m. ml}^{-1}$. After incubation of protein with cell extracts for 1 h at 37°C , an equal volume of SDS sample buffer ($0.125\ \text{M}$ Tris-HCl, pH 6.8, 10% 2-mercaptoethanol, 4% SDS¹²) added and mixture frozen until SDS gel electrophoresis. SDS gel electrophoresis: Proteins were analysed on 12.5% polyacrylamide slab gels¹³ containing 0.1% SDS using a discontinuous buffer system¹². Proteins, in sample buffer, were denatured by heating samples in boiling water for 2 min. Samples were loaded on to gels and electrophoresis carried out at a constant current of 15 mA for 4 h. Gels were fixed in 30% TCA, stained with 0.1% Coomassie blue in 30% TCA, and destained in 7.5% acetic acid. Gels were dried and autoradiograms taken. ^{35}S -labelled P32 preparations: *E. coli* B^F grown to $4 \times 10^8\ \text{cells ml}^{-1}$ in 200 ml low sulphate M9 medium (sulphate concentration $5 \times 10^{-5}\ \text{M}$) was infected; after 8 min superinfected for a total multiplicity of ten, with T4 *amBL292* (gene 55) a mutant which overproduces P32 (ref. 15). At 10 min ^{35}S -sulphate (final concentration $30\ \mu\text{Ci ml}^{-1}$) was added. After 90 min cells were collected and frozen. Purification of ^{35}S -P32 was same as for non-radioactive P32, except DEAE-cellulose chromatography omitted. Non-radioactive P32 added to final concentration $200\ \mu\text{g ml}^{-1}$ to act as carrier. Non-radioactive P32 preparations: *E. coli* B^F grown to $4 \times 10^8\ \text{cells ml}^{-1}$ in M9A medium was infected as described above. After 90 min cells collected and frozen until needed. Purification of P32 as described previously¹; 50 g cells yielded about 35 mg purified P32. Undigested P32 (both radioactive and non-radioactive) used in this experiment and those in Fig. 3 contained a small amount of P32* and three additional bands between the position of P32*-II and P32*-I (see text). These three minor components were absent when P32 was prepared using DNase pretreated with diisopropylfluorophosphate (DFP) followed by hydroxylapatite column chromatography to remove contaminating chymotrypsin B (ref. 7); therefore, they probably are intermediates of P32 digestion by chymotrypsin B to produce P32*. Cell extracts: Cultures (100 ml) *E. coli* B^F in M9A medium grown at 37°C to $5 \times 10^8\ \text{cells ml}^{-1}$ infected with T4 *amB17* (gene 23) or *amN90* (gene 21) and superinfected 5 min later for a total multiplicity of eight. Cells collected by low speed centrifugation 30 min after infection. Cell pellets suspended in 5 ml sonication buffer¹ and sonicated. After high speed centrifugation to remove cell debris, supernatants used to treat ^{35}S -P32. Uninfected cell extract prepared in same way.

P32*-II and -III appeared. (The position of P32*-II after electrophoresis in SDS gels was too close to that of P32 to detect a small amount of P32*-II in the presence of a large excess of P32. Therefore, the fact that P32*-I was seen first does not mean that P32*-II was produced after P32*-I, but rather P32*-II was not seen until the amount of P32 was reduced.) With further increases in enzyme concentration and/or reaction temperature, P32 then P32*-II and then P32*-I disappeared. Several examples showing each stage of hydrolysis are presented in Fig. 3.

Pepsin was least efficient of the three proteinases probably because it was used at a suboptimal pH; $1\ \mu\text{g ml}^{-1}$ was

required to produce a small amount of P32*-I (column 2, Fig. 3). With higher concentrations of pepsin, stage *c* (Fig. 2) hydrolysis was reached but hydrolysis did not proceed beyond this point. Chymotrypsin with an activity midway between that of pepsin and trypsin produced any stage of hydrolysis indicated in Fig. 2 with $1\ \mu\text{g ml}^{-1}$ producing stage *c* as shown in column 4 of Fig. 3. Trypsin, with the strongest proteolytic activity of the three proteinases, produced stage *c* and stage *d* hydrolysis at concentrations of 0.1 and $1\ \mu\text{g ml}^{-1}$, respectively. Chymotrypsin and trypsin at concentrations higher than $10\ \mu\text{g ml}^{-1}$ and at 37°C were able to digest P32*-III to even shorter peptide fragments.

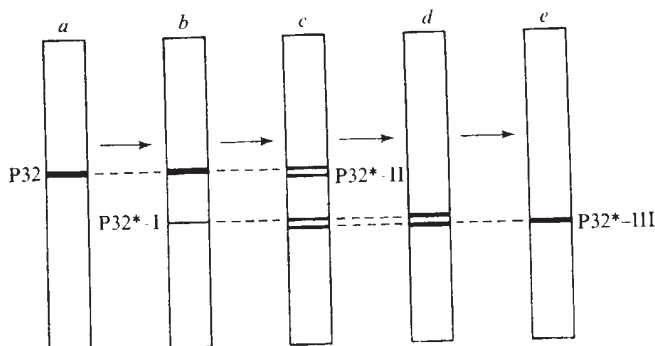
Affinity of proteolysis products of P32 for single-stranded DNA-cellulose

Affinity of the limited hydrolysis products for single-stranded DNA was examined by chromatography on single-stranded (calf thymus) DNA-cellulose columns. Limited digests containing all four components (P32, P32*-I, -II and -III) in 50 mM NaCl were loaded on columns of single-stranded DNA-cellulose and eluted off with increasing concentrations of NaCl (Table 1). Regardless of which proteinase was used for digestion, products with the same molecular weights behaved the same way. All four species adsorbed at 50 mM NaCl. Both P32 and P32*-I showed strong binding to the single-stranded DNA with elution of either requiring a concentration of NaCl higher than 0.6 M. On the other hand both P32*-II and P32*-III showed a drastic loss of affinity as both eluted off the column with 0.4 M NaCl.

Affinity of chymotrypsin-digested products for native T4 DNA-cellulose

It has been shown that P32* can denature T4 DNA⁵. P32* bound strongly to duplex T4 DNA-cellulose, whereas P32 did not (J.H. and C. Brack, unpublished). Presumably P32* is also able to denature duplex T4 DNA bound to cellulose and then bind to single-stranded regions. Binding of P32 and the limited hydrolysis products of chymotrypsin digestion to native T4 DNA-cellulose was examined to see if the products showed affinity to T4 DNA-cellulose as P32* did. Chymotrypsin was used because its activity is midway between that of trypsin and pepsin (Fig. 3). Digests containing all four components were loaded on to columns of T4 DNA-cellulose in 5 or 25 mM NaCl at 4 or 20°C . Results are summarised in Table 1b. In these conditions neither P32 nor P32*-II showed any binding to T4 DNA-cellulose. P32*-I and P32*-III both bound in the four sets

Fig. 2 Diagrammatic representation of position of limited hydrolysates produced by chymotrypsin, trypsin or pepsin, after electrophoresis on SDS gels. *a*, Untreated P32; *b-e*, limited proteolysis products with increasing enzyme substrate ratio or with increase of reaction temperature.



of conditions listed above. P32*-III eluted at NaCl concentrations of 0.1–0.2 M. P32*-I had stronger binding, as a NaCl concentration of 0.4 M was necessary for elution. This strong binding of P32*-I is similar to the binding reported earlier for P32* (ref. 5). The implication is that P32*-I is capable of denaturing T4 DNA and binding cooperatively to it. The weaker binding of P32*-III suggests that it is binding in a different manner from P32* or P32*-I.

Model for limited proteolysis (Fig. 4)

Regardless of proteinases used the products were indistinguishable by SDS gel electrophoresis and chromatography of DNA–cellulose, yet these proteinases each have a different specificity for the bond they hydrolyse: trypsin is specific for the carboxyl group of lysyl and arginyl residues; chymotrypsin for the carboxyl group of tryptophanyl, phenylalanyl and tyrosyl residues; pepsin for the carboxyl group of tryptophanyl, phenylalanyl, tyrosyl, methionyl, and leucyl residues. Therefore, we propose that because of the three-dimensional structure of the native P32 molecule there are only two regions exposed to proteinase attack. Both of these regions contain one or more of the bonds specific to their respective proteinase and these bonds are proximal to one another.

Proteolysis within one of the two regions removes a peptide (or peptides) of molecular weight 8,000 to produce P32*-I. We call it A peptide for simplicity, until further investigation can establish whether one or more than one peptides are removed. P32*-I produced by chymotrypsin cannot be exactly the same as P32*-I produced by trypsin. Their molecular weights and their DNA–cellulose-binding properties, however, are close enough so that they are indistinguishable on SDS gel electrophoresis or DNA–cellulose column chromatography. Similarly, proteolysis within the other region removes a peptide (or peptides) of molecular weight approximately 1,000 (B peptide) to create P32*-II. Hydrolysis at both regions, to remove A and B peptides, produces P32*-III. Loss of A peptide did not decrease affinity to single-stranded DNA. It did, however, increase affinity to duplex (T4) DNA in both P32*-I and P32*-III. Removal of B peptide resulted in decreased affinity for single-stranded DNA–cellulose. Removal of both A and B peptides, as in P32*-III, resulted in decreased

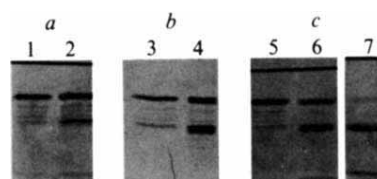


Fig. 3 Autoradiogram showing position of ^{35}S -labelled P32 limited hydrolysates after electrophoresis. *a*, Column 1, untreated P32; column 2, P32 treated with $1\text{ }\mu\text{g ml}^{-1}$ pepsin; *b*, column 3, untreated P32; column 4, P32 treated with $1\text{ }\mu\text{g ml}^{-1}$ chymotrypsin; *c*, column 5, untreated P32; column 6, P32 treated with $0.1\text{ }\mu\text{g ml}^{-1}$ trypsin (TPCK-treated); column 7, P32 treated with $1\text{ }\mu\text{g ml}^{-1}$ trypsin. Digestion was in sonication buffer¹ at 4°C for 1 h. Equal volume SDS sample buffer added and samples heated in boiling water for 2 min before electrophoresis. For preparation of ^{35}S -labelled P32 and electrophoresis, see Fig. 1.

affinity for single-stranded DNA but increased affinity for duplex DNA.

Model for control of P32 activity at replication fork

Both DNA replication and recombination, in which gene 32 participates, are extremely complex reactions involving a multitude of different proteins. In such systems, it is probable that proteins function as complexes and that protein–protein interactions are the major factor in control of each component's activity.

Efforts to reconstruct the T4 DNA replication apparatus from purified components have been successful⁸. This system requires six T4 gene products (32, 41, 43, 44, 45, and 62) all of which are necessary for *in vivo* replication. Isolation and purification of these six has been accomplished. Because components interact synergistically with one another they probably form a complex of unique structure. This complex has a propagation reaction *in vitro* which closely mimics that found *in vivo*. All six must be present *in vitro* for extensive synthesis by strand displacement on duplex DNA templates (leading strand synthesis) and for *de novo* DNA chain initiation on single-stranded DNA templates (lagging strand synthesis), even though the individual components may not have the same activity (or any activity at all) outside the complex⁸.

Table 1 Column chromatography of P32 and its limited proteolysis products on DNA–cellulose

Protein	Molecular weight ($\times 10^{-3}$)	Expt <i>a</i> —Chromatography on denatured DNA–cellulose					Expt <i>b</i> [†] —Chromatography on native DNA–cellulose			
		NaCl* (M)	Adsorption		Elution		Adsorption		Elution	
			0.05	0.4	0.60	2.0	0.005 or 0.025	0.1	0.2	0.4 or more
P32	35		+	—	—(†)	+	—			
P32*-I	27		+	—	—(†)	+	+	—	—(†)	+
P32*-II	34		+	+			—			
P32*-III	26		+	+			+	+		

* In buffer *b*: 20 mM Tris-HCl, pH 8.1, 1 mM Na₃EDTA, 1 mM β -mercaptoethanol and 10% glycerol.

† A small fraction occasionally eluted at this concentration.

‡ T4 DNA–cellulose was irradiated with ultraviolet light¹⁷. Unirradiated T4 DNA–cellulose gave similar results (J.H. and C. Brack, unpublished).

^{35}S -labelled P32 (0.84 mg , 2.4×10^5 c.p.m. ml^{-1}) in buffer *b* was treated with $1\text{ }\mu\text{g ml}^{-1}$ (freshly dissolved) enzyme at 4°C for 1 h. Either TPCK (10^{-3} M), for chymotrypsin-treated samples, or tosyl-lysyl-chloromethyl-ketone (TLCK, 10^{-3} M), for trypsin-treated samples, was added. After 15 min more incubation to allow time for complete proteinase inhibition, solution was loaded on DNA–cellulose column. Experiment *a* combined results of four separate single-stranded calf thymus DNA–cellulose columns¹⁶, all run at the same time. Products for each column were produced by different proteases—trypsin, chymotrypsin or pepsin. Digests containing 0.05 M NaCl were applied, columns were washed with 2 ml 0.05 M NaCl and proteins eluted off with 1 ml each of following concentrations NaCl: 0.4, 0.60 and 2.0 M. Each fraction (0.5 ml) was monitored for radioactivity, and analysed on SDS gel electrophoresis. Experiment *b* combined results of chromatography of limited hydrolysis products of chymotrypsin on four separate native T4 DNA–cellulose columns (ultraviolet-irradiated¹⁷). Columns were loaded at 5 or 20°C with digests containing either 0.005 or 0.025 M NaCl. Proteins were eluted with following concentrations NaCl: 0.1, 0.15, 0.2, 0.4 and 0.60 M. (In all four cases products with the same molecular weight behaved the same way, regardless of loading temperature or NaCl concentration.)

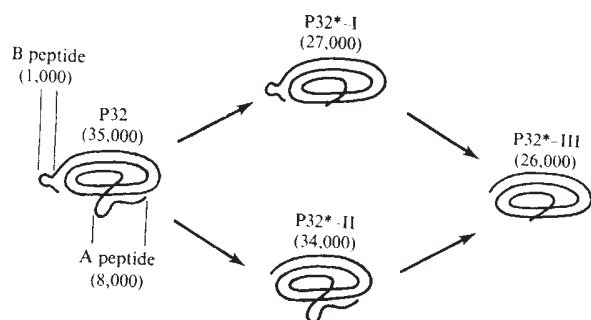


Fig. 4 Model for limited proteolysis of P32.

Two types of protein-protein interaction have been reported for P32; intra-action, that is, self-association¹; interaction, that is, an affinity for T4 DNA polymerase². Self-association, as first described, was regarded as related to the cooperative binding of P32 to DNA¹. Affinity for T4 DNA polymerase is related to P32 stimulation of polymerase activity³. When P32 molecules are within the replication machinery, there would undoubtedly be greater probability of increasingly complex intra- and interactions.

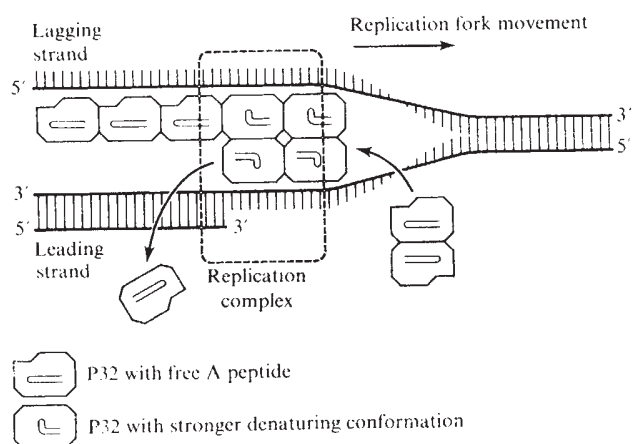
High susceptibility of A and B peptides to hydrolysis by various proteinases implies that these peptides are easily accessible to other proteins. Removal of either or both resulted in modification of the binding characteristics. Based on the data presented in this report, we suggest that A and B peptides are involved in protein-protein interactions and through such interactions the activity of P32, *in vivo*, is controlled.

With the removal of A peptide, affinity of P32*-I and P32*-III for duplex T4 DNA is greatly increased. This suggests that A peptide might be involved with the DNA-binding site in that its presence or absence greatly influences denaturing ability of the protein.

This involvement could be a direct spatial relationship to the DNA-binding site. It could also be that the interaction of A peptide with another protein leads to a conformational change in P32 resulting in a change in binding ability. It may be that, *in vivo*, A peptide limits the mode of action of P32 to that of a renaturing protein. This could have profound biological significance because the presence of too strong a denaturing protein could cause disastrous uncontrolled melting of the chromosome.

Based on this consideration we developed the following model: The role of A peptide is to localise unwinding of duplex DNA in advance of the replication fork (or within the replication complex) as shown in Fig. 5. P32, *in vivo*, acts in a renaturing or denaturing mode as regulated by A peptide and the environment in which A peptide is

Fig. 5 Model for role of P32 in DNA replication.



placed. When P32 is outside the replication complex, A peptide exerts an inhibitory influence on denaturing, and P32 reacts in a renaturing mode. Proteolytic removal of A peptide *in vitro* produces P32*-I with more potent denaturing activity than the original P32. In theory, the same, or even stronger, activity could be reversibly produced *in vivo* by the interaction of A peptide with the replication complex, which generates an environment in which denaturation of the duplex DNA should take place.

We have adopted part of a published model of self-association of P32 as follows: P32 dimer is the basic DNA binding unit in physiological conditions; the dimers insert themselves between two strands and bind to the phosphodeoxyribose backbone holding the bases in an outward position¹⁰. The advantage of this model is that with the bases held partially separated from each other, they are free to hydrogen bond with the incoming nucleotide precursors. After the replication complex (engaged in leading strand synthesis) has passed over the P32 molecules, which have been holding the duplex DNA apart, A peptide no longer interacts with the replication complex and again asserts its inhibitory influence. Thus P32 no longer prevents hydrogen bonding between the newly made leading strand and its template. P32 remains bound to the opposite strand (Fig. 5) which then acts as the template for lagging strand synthesis. P32 is required for lagging strand synthesis, and it does not fall off the single strands until lagging strand synthesis takes place.

Our data have shown that B peptide is involved in the tight binding of P32 to single-stranded DNA. There could be at least three different reasons for this: P32 has more than one DNA-binding site and B peptide contains some of them; loss of B peptide induces a conformation change unfavourable to tight binding; or B peptide is related to the cooperativity of self-association of P32.

Extensive studies of self-association by sedimentation equilibrium has led to the proposal that there are at least two types of self-association: association to dimers, which are resistant to high salt concentration and the presence of Mg^{2+} and association of these dimers to higher molecular weight aggregates in conditions of low ionic strength and high protein concentration^{10,11}. An earlier model suggested that in physiological conditions P32 exists as dimers and association of these dimers is related to cooperative binding¹⁰. In a later model this idea was modified to suggest that binding to DNA occurs after P32 has aggregated to a higher molecular weight species¹¹. In our more dynamic model (Fig. 5) the change of P32 to a stronger denaturing protein could include increased affinity between dimers. This would result in a contiguous binding site at the front of the replication complex, attractive to dimer-unit binding.

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Body weight, diet and home range area in primates

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Primates show a strong positive relationship between body weight and home range area. Dietary habits also influence home range area. Folivorous primates occupy smaller home range areas for their body weight than do frugivores and omnivores. Primates generally require smaller home range area per individual than solitary terrestrial mammals, but primates living in social groups have much larger total home range than individual solitary mammals. This trend may necessitate higher expenditures of energy in food-gathering or modifications in movement patterns.

NUMEROUS studies during the past decade have shed light on relationships between various aspects of primate ecology¹⁻⁵, but as yet there has been no quantitative summary of data on home range area of primates that takes into account the effects on spatial needs of both body size and diet. A strong positive relationship exists between body weight and home range area in lizards⁶, birds^{7,8} and solitary mammals⁹, while McNab⁹ showed for mammals and Schoener⁸ for birds that feeding habits also have a predictable effect on the home range area of many species. Clutton-Brock¹⁰ pointed out that rather small differences in feeding ecology may affect markedly the ranging patterns of primates. Here we analyse data on the body weight, diet and home range area of 36 primate species, and demonstrate a relationship among these variables.

The home range data in this paper are taken from diverse sources and are undoubtedly not perfectly comparable (Table 1). Most body weights were obtained by averaging adult male and adult female body weights, and thus do not take into account interspecies differences in sociometric sex ratio or the fact that many group members may be subadult. Average weights in general are probably overestimated, particularly in the case of large terrestrial omnivores like the baboon (*Papio*), in which sexual dimorphism is marked. For most species, however, sufficient data are not available, either on group composition or weight variation with age and sex, to permit calculation of more accurate average weights. In addition, rather large (for example, twofold) errors in estimating body weight will cause a shift in log weight of < 10% of the entire range of data. We believe that errors introduced by differences in techniques of home-range estimation and those resulting from inaccuracies in calculating weight are not sufficient to be critical to our general conclusions.

Diet was determined from the sources of home-range information whenever possible, supplemented by material summarised by Jolly³ and Kay¹¹. We classify as folivores those species that seem to depend on foliage, mature or immature, as their staple diet. Frugivores are primarily fruit eaters, taking only a small amount of foliage and little or no animal protein. Species eating roughly equal proportions of both foliage and fruit and little or no animal protein are classified as generalist primary consumers, and those which seem to actively seek out and probably to depend on animal protein in the diet are

classified as omnivores. Obviously there are degrees of folivory, frugivory and omnivory and no category should be regarded as definitive; they are our best estimate of the dietary preferences of individual species. Terrestriality and arboreality, too, are points on a continuum, and assignments should not be regarded as absolute. Generally, unless a species is known to consistently travel or forage on the ground each day, we considered it arboreal.

Regressions of $\log_{10}(\text{home range})$ on $\log_{10}(\text{body weight})$ were fitted by the method of least squares. Significance of the regressions was determined by calculating Student's *t*-statistic for the deviation from zero of the slopes. Our criterion for significance is the 0.05 level of probability. Spearman rank correlation coefficients ρ for the untransformed data served as an additional confirmation of correlation between variables. The tests were in agreement in all cases.

Individual home range allocation

Figure 1 shows home range area divided by the number of individuals per group (HR_i) as a function of body weight. The species are listed in Table 1. There is a strong positive relationship between body weight and home range area in primates ($r^2 = 0.44$; $t = 5.14$, $P < 0.005$). The slope of the regression line is 0.79, compared with 0.63 found by McNab⁹ for temperate-zone solitary terrestrial mammals. The figure also shows each species placed in one of the four dietary categories. The more folivorous primates show a strong tendency toward a smaller HR_i than the frugivorous and omnivorous species. Eight of twelve terrestrial primates have a larger HR_i than might be expected for their weight, whereas arboreal primates show a wider scatter both above and below the regression line.

McNab divided mammals on the basis of food habits into 'hunters' (discrete particle feeders, for example, granivores, frugivores, insectivores, carnivores) and 'croppers' (browsers and grazers). Frugivorous and omnivorous primates resemble McNab's hunters in feeding habits, whereas the folivores are more like his croppers. Figure 2 shows primates grouped as hunters (frugivores and omnivores) or croppers (folivores), compared with relationships predicted by McNab for hunters and croppers. Generalist primary consumers are not considered in this comparison because they are not readily assigned to either category, and because the small sample size and weight range involved severely limits the value of any statistical treatment. The trend in this group seems, however, to resemble that among croppers more than hunters.

Both of the subgroups considered show stronger relationships between body weight and HR_i than do primates in general (for primate hunters, $r^2 = 0.66$, for croppers $r^2 = 0.49$). The slope of the primate hunter line, 0.83, is close to that calculated for hunters by McNab, although the expected values are only about half those predicted by McNab. The slope of the line for primate croppers is 1.06, steeper than that of McNab's cropper line or the primate hunter line but not significantly different from either. Analysis of covariance of $\log HR_i$ with feeding category and body weight for primate hunters and croppers using a randomised groups design¹², shows that adjusted group means are significantly different ($F = 69.1$, $P < 0.01$), with croppers having substantially smaller home ranges. Primate

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croppers also tend to occupy smaller HR_i than do solitary, non-primate croppers up to $\sim 2 \times 10^4$ g.

Values of HR_i should give some idea of the relative amount of space required to provide adequate resources for individuals of a species. What factors determine HR_i ? Clearly body size is very important; once this has been accounted for other influences become more apparent. Folivory is associated with allocation of relatively small areas per individual in arboreal primates. Most of these animals are at least partially croppers in the sense of McNab⁹, and are likely to have concentrated resources available. Also, the height of the tree canopy may have an especially marked effect on the size of the home range of leaf eaters. This third dimension adds to the density of resources per unit area and to the actual distance travelled in space per unit area¹³⁻¹⁵. In addition, McNab has recently suggested (personal communication) that rates of metabolism of arboreal folivorous mammals may be below those expected for mammals in general, so conceivably the energy requirements

of many folivorous primates are relatively low. All of these explanations, however, entail difficulties, some of which are discussed below.

Frugivores tend to have larger HR_i than folivores. Typical tropical forests are highly heterogeneous in species composition¹⁶⁻¹⁸. Fruiting trees are often widely dispersed in time and space. Thus most frugivores depend on clumped, unstable food sources, a resource pattern which has been associated with a relatively large home range area¹⁰. Also, there is some reason to suspect that frugivory is correlated with high rates of metabolism¹⁹.

Among generalist primary consumers HR_i may vary with the proportion of fruit and leaves eaten. For example, in Guanacaste, Costa Rica, where it feeds $\sim 85\%$ of the time on foliage, *Alouatta palliata* has a small HR_i (K. Glander, personal communication) when compared with the same species on Barro Colorado Island where it spends approximately equal amounts of time feeding on foliage and fruit (K. M., personal

Table 1 Species, home range, and source of data

Species	Weight* (g)	HR_i (ha)	HR_t (ha)	Class†	Reference	Remarks
Prosimians						
<i>Galago demidovii</i>	60	0.80	0.80	AO	41	♀♀ only
<i>Lemur catta</i>	2,300	0.29	5.7	TP	42	
<i>L. catta</i>	2,300	0.44	7.4	TP	21	
<i>L. fulvus rufus</i>	2,370	0.10	0.88	AL	21	
<i>Lepilemur mustelinus</i>	650	0.24	0.24	AL	43	
<i>Microcebus murinus</i>	60	0.20	0.20	AO	44	♀♀ only
<i>Propithecus verreauxi</i>	3,800	1.8	9.0	AL	45	
<i>P. verreauxi</i>	3,800	0.48	2.4	AL	42	
Cebioidea						
<i>Alouatta palliata</i>	6,875	0.76	9.9	AP	K. Glander, personal communication	85% folivorous
<i>A. palliata</i>	6,875	2.8	45	AP	K. Milton, personal observation	50% folivorous
<i>A. seniculus</i>	7,250	0.38	3.2	AP	46	
<i>Ateles belzebuth</i>	5,800	14	320	AF	47	
<i>Callicebus moloch</i>	600	0.15	0.44	AO	48	
<i>Cebus capucinus</i>	3,100	6.1	86	AO	39	
<i>Saimiri oerstedii</i>	840	0.74	17	AO	49	
<i>Saguinus geoffroyi</i>	550	2.4	8	AO	50	HR_i estimated from maximum linear range
Cercopithecoidea						
<i>Cercocebus albigena</i>	7,900	1.2	21	AF	51	
<i>C. albigena</i>	7,900	9.0	140	AF	52	
<i>Cercopithecus aethiops</i>	3,800	1.4	40	TO	53	
<i>C. mitis</i>	6,000	2.8	70	AO	R. Rudran, personal communication	Weight estimated by H. Schlichte, personal communication
<i>C. talapoin</i>	1,130	4.0	400	AO	3	
<i>Colobus badius</i>	8,000	2.5	100	AL	10	
<i>C. guereza</i>	8,000	2.7	20	AL	10	
<i>C. guereza</i>	8,000	1.8	14	AL	54	
<i>Erythrocebus patas</i>	9,260	170	5,200	TO	55	
<i>Macaca mulatta</i>	7,300	7.9	200	TO	56	
<i>M. radiata</i>	5,140	8.9	520	TO	57	
<i>M. sinica</i>	5,130	3.0	38	TF	15	
<i>Nasalis larvatus</i>	15,100	6.5	130	AL	58	
<i>Papio anubis</i>	21,400	10	470	TO	59	
<i>P. anubis</i>	21,400	60	2,300	TO	1	Gallery forest
<i>P. cynocephalus</i>	15,100	62	2,500	TO	31	Savanna
<i>P. ursinus</i>	20,600	24	1,100	TO	1	
<i>Presbytis cristatus</i>	6,300	0.63	20	AL	60	
<i>P. entellus</i>	17,200	12	630	TL	34	Kankori site
<i>P. entellus</i>	17,200	34	640	TL	34	Orcha site
<i>P. entellus</i>	17,200	0.87	19	TL	61	Dwhar site
<i>P. entellus</i>	17,200	0.56	14	TL	15	Polonnaruwa, Ceylon, site
<i>P. johnii</i>	8,170	18	160	AL	62	
<i>P. senex</i>	5,980	3.0	12	AL	15	
Hominoidea						
<i>Hylobates lar</i>	5,540	11	45	AF	63	
<i>H. lar</i>	5,540	34	100	AF	20	
<i>Gorilla gorilla gorilla</i>	145,000	150	3,300	TL	64	
<i>Pan troglodytes</i>	40,700	23	1,100	TF	35	
<i>Pongo pygmaeus</i>	36,500	65	65	AP	65	♀♀ only
<i>Symphalangus syndactylus</i>	10,500	10	42	AP	66	

* Body weights taken from Kay¹¹, Napier and Napier¹⁰, or from same source as home range data.

† Indicates habitat and feeding class: A, arboreal; T, terrestrial; L, folivore; F, frugivore; P, generalist primary consumer; O, omnivore.

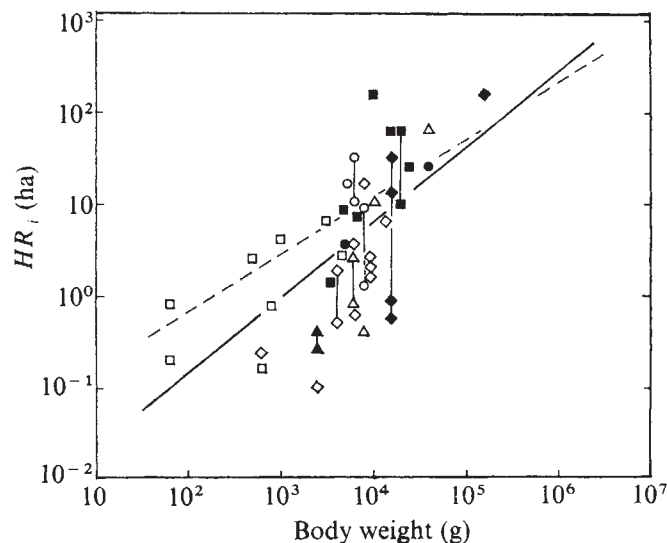


Fig. 1 Home range per individual as a function of body weight. Vertical lines connecting similar symbols indicate data from different studies of the same species. Values for the log of the mean home range for the species were substituted for these points in all calculations here and in Figs 2 and 3. Heavy solid line is least squares regression of $\log_{10} HR_i$ on $\log_{10}(\text{body weight})$ for primates ($\log HR_i = 0.79 \log BW - 2.32$). Dashed line is regression calculated by McNab⁹ for solitary, temperate zone mammals. Open symbols designate arboreal species, solid symbols terrestrial species. $\diamond$, folivores; $\circ$, frugivores; $\triangle$, generalist primary consumers; $\square$, omnivores.

observation). The siamang (*Symphalangus*), which spends ~ 50 per cent of its annual feeding time eating foliage, has a smaller home range than the gibbon (*Hylobates*), a smaller primate that seems to be more frugivorous^{18,20}. *Lemur catta*, a generalist primary consumer that was studied at two sites, had a smaller home range where it was more frugivorous. In the study site where it ate less fruit and had a larger home range, however, it was sympatric with another lemur species, *L. fulvus rufus*, that also ate some fruit. Further, *L. catta* was much more frugivorous than *L. fulvus rufus* and had a much larger HR_i in the habitat where the two species were sympatric, even though they have about the same body weight²¹.

Arboreal omnivores generally occupy a large HR_i for their body weight, as large, in fact, as terrestrial omnivores. This suggests that arboreality *per se* does not reduce the area required to provide adequate nutritional resources for omnivores. Arboreal omnivores probably depend more heavily on insects in the diet than the larger, terrestrial omnivores, and may, therefore, occupy a slightly higher trophic level than their terrestrial counterparts. Nevertheless, solitary arboreal squirrels, which are also discrete particle feeders (but are certainly primarily herbivorous), have HR_i comparable to the primate HR_i of this group^{22,23}.

Terrestrial primates as a group tend to have an HR_i somewhat larger than expected for their body weight. Some of these species live in arid regions that probably have lower densities of resources per given area². The patas monkey (*Erythrocebus patas*) is an extreme case and it has a very large HR_i . Home range data are not available for two other arid country species, the hamadryas baboon (*Papio hamadryas*) and the gelada (*Theropithecus gelada*), but information on daily range indicates that they also cover very large home range areas³. Except for these arid land forms, it is not clear that terrestriality in itself is correlated with large HR_i . Terrestrial folivores do have larger HR_i values than arboreal folivores, but the differences may arise simply from their larger size.

Primate hunters and croppers generally have lower values of HR_i than predicted for temperate zone solitary mammals of corresponding feeding habits. The small individual home ranges may reflect: (1), the fact that some hunters may some-

times graze or browse; (2), the high productivity of many tropical areas compared to mesic temperate zone habitats; (3), the additional resource space provided by the depth of the forest canopy; or (4), the greater efficiency of utilisation of area as a result of being social. All of these factors may contribute in particular cases. Certainly some frugivores, and very probably some terrestrial omnivores, eat enough leaves or grass to make a substantial impact on their resource area requirements. The year-round high productivity of many tropical regions may be important, but much of this productivity may be unavailable as animal food^{16,24-26}. The idea that the greater vertical extent of the resource space for arboreal species should reduce the HR_i required is attractive *a priori*, but it is not clear why this should apply to folivores only. Some of the possible effects of sociality are discussed below.

We have not tried to take into account the effect on home range area of overlap between conspecific groups, although one might expect that HR_i as well as total home range would be larger in species with greater overlap. The little data available suggest this may be true, since most species with non-overlapping ranges (for example, *Callicebus moloch*, *Symphalangus syndactylus*, *Colobus guereza*, *Propithecus verreauxi*), occupy home range areas smaller than or equal to the predicted values.

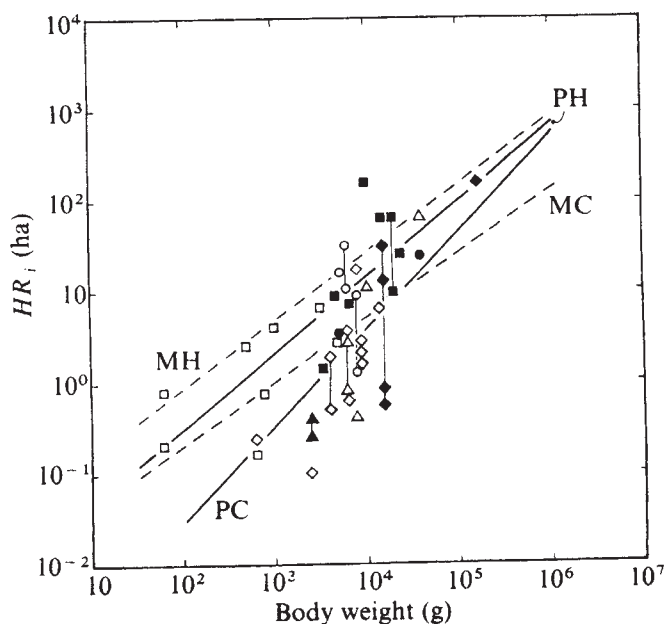
Total home range

Figure 3 shows total group home range (HR_g) as a function of body weight. The slope of the regression is significant ($r^2 = 0.56$; $t = 6.54$, $P < 0.005$). Much of the deviation from the regression in Fig. 3 may be due to variation in group size, since there is a strong correlation between group size and HR_i ($\rho = 0.67$; $P < 0.0001$) irrespective of body weight.

Broad patterns of variation in total group home range are generally similar to those noted for HR_i . Both folivores and generalist primary consumers tend to have smaller HR_i than frugivores and omnivores. To a greater extent than HR_i , HR_g of terrestrial species tends to be higher than expected.

Perhaps the most interesting aspect of Fig. 3 is that HR_i for many group-living primates is far larger than the home range of solitary animals of similar body weight, although solitary

Fig. 2 Influence of diet on HR_i . Heavy solid lines are regressions of $\log_{10} HR_i$ on $\log_{10}(\text{body weight})$ for frugivores and omnivores (primate hunters, PH; $\log HR_i = 0.83 \log BW - 2.17$) and folivores (primate croppers, PC; $\log HR_i = 1.06 \log BW - 3.66$) separately. Generalist primary consumers are excluded from the calculations. Dashed lines are regressions calculated by McNab⁹ for solitary, temperate zone hunters and croppers (MH, hunters; MC, croppers). Species symbols as in Fig. 1.



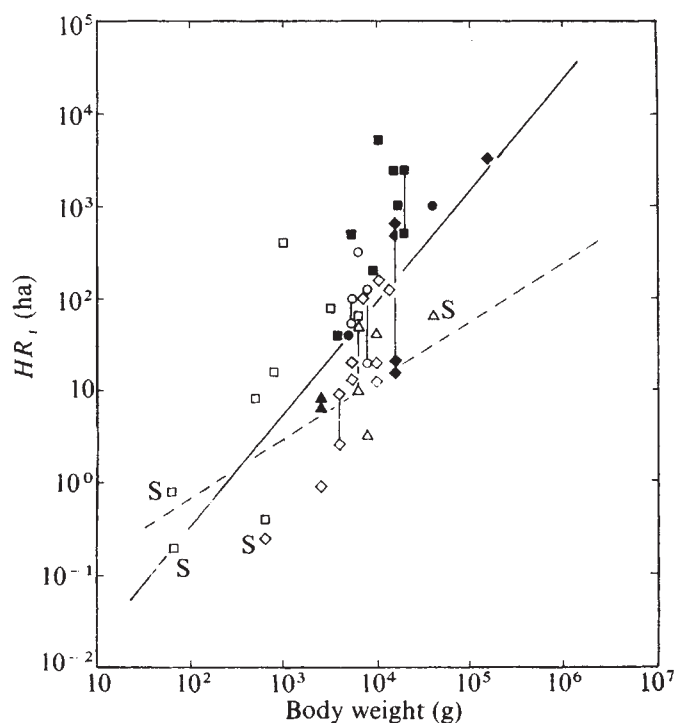


Fig. 3 Total home range per group as a function of body weight. Symbols as in Fig. 1. S indicates solitary foragers. Regression equation for primates is $\log HR_g = 1.23 \log BW - 2.86$.

primates occupy home ranges similar to those of other mammals. If, within a given time interval, the primate group covers the same proportion of its home range using the same movement patterns as a solitary mammal, the individual primate may have to expend much more energy in travel than solitary mammals of similar size. How can social primates afford or avoid the higher cost of obtaining their resources? We can suggest three possibilities which are not mutually exclusive and the second and third may be related. First, it is possible that locomotion is a negligible portion of the energy budget. This seems unlikely in view of what is known of the metabolic cost of locomotion²⁷. Second, it may be that foraging as a group increases food-finding efficiency enough to compensate for increased travel costs. Undoubtedly energy required for foraging is reduced by a shared pool of traditional knowledge about resource timing and location and travel routes²⁸⁻³⁰. Third, and probably most important, the pattern of movement within the home range may differ consistently between social and solitary animals. For example, a group may traverse a large area over a broad front while each individual moves over a fairly straight course^{31,32}. A solitary animal would have to zig-zag back and forth many times to cover the same area. A social animal can feed on smaller food patches in its path and cue other group members to the location of rich patches if the latter are encountered. Some species of iguanid lizards use conspecific cuing for locating rich food resources, and this is probably a time-saving method of food location³³. A similar model might apply within a group of primates, on a short time scale. As long as there is intraspecific tolerance of feeding at the same patch, straight line movement with cuing on other group members would be an efficient strategy, particularly if food is often clumped in fairly rich patches.

Many social animals seem to move directly between known resource patches rather than depending on chance encounters. Solitary foragers also do this (G. G. Montgomery, personal communication), but perhaps, as suggested above, group knowledge encourages such behaviour by increasing the probability that resource patches will be accurately recognised and remembered. In addition, social animals may utilise only a small portion of their home range within a given time span, covering

the entire area only in the course of weeks or even months. Certainly many group-living primates show such behaviour (for example, *Alouatta palliata* (K. M., personal observation); *Presbytis entellus*³⁴; *Pan troglodytes*³⁵; *Papio cynocephalus*³¹). Data on daily movements within their home range are scanty for solitary mammals. Red foxes generally cover most of their range each night³⁶, while raccoons³⁷, rabbits³⁸, and anteaters (*Tamandua* (G. G. Montgomery, personal communication)) do so in a few days to a week. Certain social primates traverse much of their home range in a single day, at least occasionally, but except for species which defend relatively small territories, this behaviour is usually interspersed with relatively long periods in a small area (for example, *Cebus capucinus*³⁹).

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letters to nature

New map of the optical polarisation of galaxy M82

M82 is the nearest galaxy evidently containing a strong central source of energy, which is possibly similar to the nucleus of a Seyfert galaxy, and this seems to have given rise to an unusual explosive event¹. The appearance of M82 is that of a flattened irregular system seen nearly edge-on, and if a distinct nucleus exists, it is highly obscured² at optical wavelengths. Compact radio features are, however, present, and complex resolved radio and infrared sources and bright visible H II regions occupy an area $\sim 12'' \times 35''$ in the centre of the galaxy. The outer parts of the galaxy show linear optical polarisation, discovered by Elvius³. This is thought to arise from reflection in an extensive halo of dust particles of the light of the bright nucleus (or nuclear region) and of the galactic disk (for a recent discussion, see ref. 4). Consequently, observation of the polarisation can give information about the position and luminosity of the energetic nuclear region, and about the scattering medium. Outstanding questions about M82 involve the existence and nature of a compact nucleus and the morphological and evolutionary state of the whole galaxy.

The observations reported here were obtained with the 1-m telescope of the Wise Observatory, Israel, during the period February 27–March 15, 1975. The polarimetric technique is derived from that of Ohman⁵, but uses a rotating achromatic half-wave plate, and a Wollaston prism to separate orthogonally polarised beams, which are recorded simultaneously and remain in a fixed relationship to the detector. The technique and the computer programmes used in the analysis will be discussed elsewhere. The detector was an electronographic camera⁶ on loan from the Royal Greenwich Observatory to the Wise Observatory. The waveband used was B of the UBV system.

The results of the observations are shown in Fig. 1. Each measurement is indicated by a line parallel to the plane of the electric vector and proportional in length to the degree of polarisation, centred on the point observed. Each determination is made over an area $\sim 8'' \times 8''$. The map has a superimposed photograph of M82 to illustrate the relationship of the polarisation structure to the bright part of the galaxy. The photograph is from a blue plate taken on the Isaac Newton telescope by Dr S. V. M. Clube. The eight stars indicated were used for astrometric purposes.

These observations show the polarisation at more than ten times the total number of points previously observed, with a spatial resolution three to five times finer. The

Fig. 1 The linear optical polarisation of M82 in the B waveband. ★ represent fiducial stars, ◆ the centre of symmetry, —, 10% polarisation.

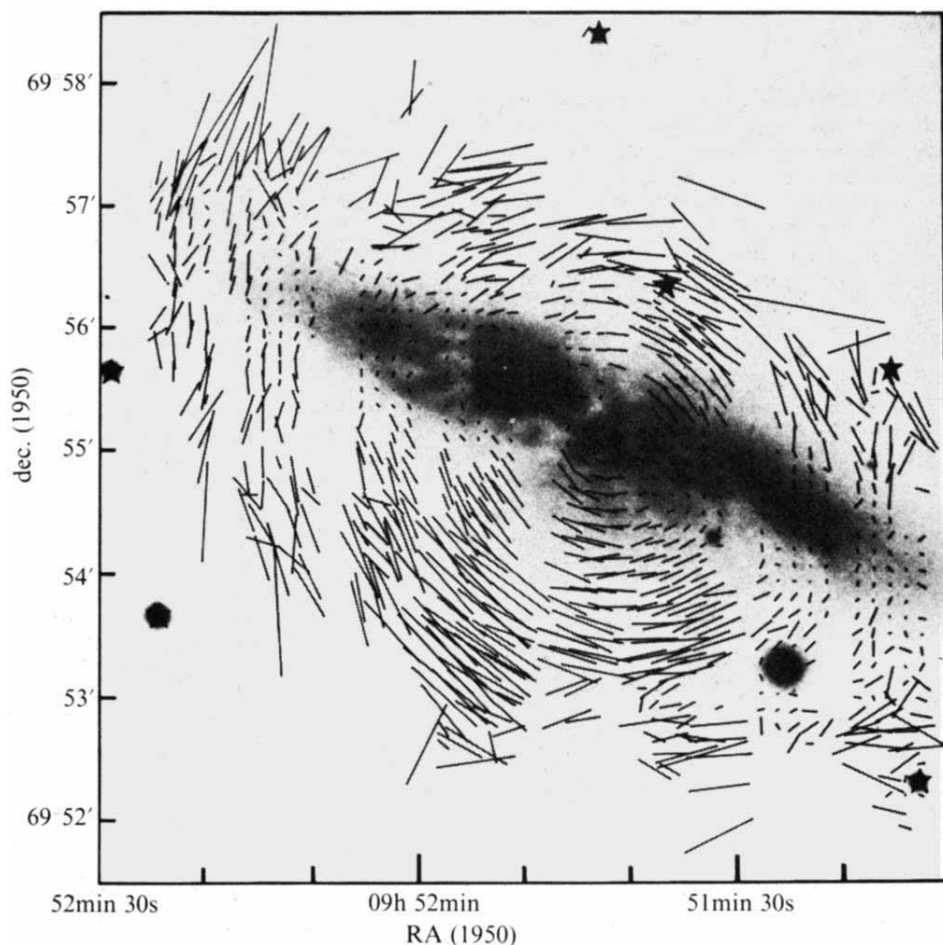


Table 1 Comparison between our measurements and published⁹ values for polarised stars

Star	Tabulated value		This work	
	<i>P</i> (%)	θ (deg)	<i>P</i> (%)	θ (deg)
HD 43384	2.75 ± 0.05	170 ± 1	2.3 ± 0.4	172 ± 5
HD 122945	0.1 ± 0.07	56 ± 18	0.3 ± 0.4	69 ± 20
HD 155528	4.6 ± 0.09	93 ± 1	4.3 ± 0.4	90 ± 3
HD 80083	0.13 ± 0.09	140 ± 17	1.4 ± 0.4	128 ± 10

results are complete over a large area of the galaxy, but as we are carrying out further work which may eventually serve to extend and improve the data, we refrain from tabulating the extensive numerical results at this stage.

The accuracy of the observations has been checked by comparison with the previous work^{3,7,8} on M82, and by observations of individual stars of known polarisation⁹. The comparison between our measurements and published values for the stars is given in Table 1. From these results, it was not considered necessary to make any corrections for instrumental effects. Also from these results and from the comparison with previous work on M82 and from our internal consistency, the standard error over most of the map, for a typical point with 15% polarisation was estimated as 2% in degree of polarisation, and 3° in position angle. These errors are comparable with previous work. The weaker alignment of the polarisation vectors seen near the edge of the map probably arises from the reduced contrast of the galaxy against the sky background, introducing a noise component. A further interpretation of these results will be given elsewhere, but a preliminary note of the more outstanding features is made here.

The completion of the two-dimensional polarisation study provides data for the construction of improved models of M82. For example, the fairly clear division of the area into regions of high and low polarisation represents a separation in depth, at least near the centre of the map. The edge-on view of the galaxy shows the near side of the disk along the central band, whereas on each side of the disk an unobscured line of sight passes through highly polarised regions directly illuminated by the bright central source, and with large scattering angles. We hope to characterise the central source and the reflecting medium by fitting models to the observational data. Some smaller contribution to the polarisation by other mechanisms is also to be expected and may be apparent in one or two features of the galactic disk in Fig. 1; these most probably arise from the properties of the disk rather than the halo material, and may show that there is some magnetic field

alignment in this irregular object. The centrally symmetric pattern enables a position to be found for the central source of radiation, following Solinger and Markert⁴. The position obtained is indicated in Fig. 1 and on a larger scale in Fig. 2, superimposed on optical and infrared contour maps, and with other interesting positions marked. The centre of symmetry may be influenced by emission from the disk as well as the nucleus of the galaxy (Solinger and Markert assume the disk is symmetrical), and it is not at present clear what difference this will make. The position obtained was, however, found to be consistent, within the indicated error, when different areas of the map were used in its derivation, and it is quite independent of previous polarisation studies.

Some previous writers have been at pains to emphasise that various central features in M82 were almost coincident, but we would point out that there is no detailed correspondence between them, such as would be expected if a true nucleus were present. The most intense radio component (cross-hatched in Fig. 2) has no special position in the infrared source, which itself is only near to the centre of symmetry. The optically brightest spot, which is sometimes assumed to be the centre in dynamical studies, is close to the centre of the infrared source but not to the other features.

The lack of an identified singular nucleus does not, however, preclude the possibility that M82 has some resemblance to a Seyfert galaxy. A compact nucleus may be present, but still unidentified, and even if no such nucleus exists, an interesting possibility arises from McCrea's theory of the Seyfert phenomenon¹⁰, which he ascribes to supernova events at the rate of perhaps one every 10 yr, in a limited volume. (No direct triggering of the events is called for, but star formation may be stimulated by previous outbursts.) In the case of M82, referring to Fig. 2, the active region of the galaxy may be ~ 300 pc in diameter, about ten times the size of a Seyfert nucleus, and the process would be much modified; however, it seems particularly appropriate for a galaxy such as M82, containing as it does a large amount of interstellar matter and regions of star formation (H II regions) within the 300 pc central active domain. A galaxy of this type, if we had a face-on view, might not qualify as a Seyfert from its spectral characteristics, but could represent the connecting link between Seyferts and normal galaxies.

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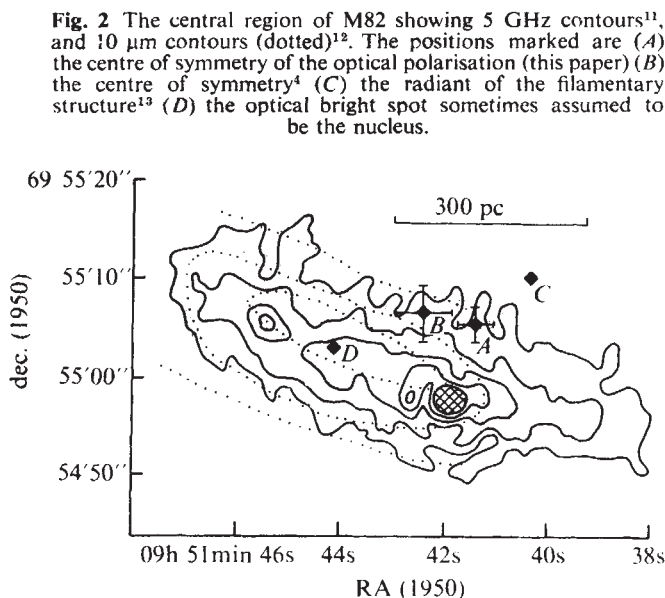


Fig. 2 The central region of M82 showing 5 GHz contours¹¹, and 10 μ m contours (dotted)¹². The positions marked are (A) the centre of symmetry of the optical polarisation (this paper) (B) the centre of symmetry⁴ (C) the radiant of the filamentary structure¹³ (D) the optical bright spot sometimes assumed to be the nucleus.

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Variations of the infrared polarisation of VY Canis Majoris

A REMARKABLE change in the infrared polarisation of VY Canis Majoris has been observed in 1974 and 1975. This star was found, in 1970, to have a large and peculiar polarisation¹. Further observations carried out in 1971 and 1972 confirm the existence of a polarisation peak at $1.6\ \mu\text{m}$ and a rapid rotation of the position angle of polarisation from visible to infrared wavelengths^{2–5} (see Fig. 1). The polarisation declines rapidly with wavelength from ultra-violet to infrared, having a dip near $1\ \mu\text{m}$, and rising further in the infrared, with peak at $\lambda=1.6\ \mu\text{m}$. Corresponding to the rapid decrease in the polarisation, the position angle rotates by about 90° . Our latest observations show that the hump at $1.6\ \mu\text{m}$ has disappeared.

Our original results suggested that the polarisation could be resolved into two components, visible and infrared polarisations, and the visible polarisation has since been

Fig. 1 The wavelength dependence of the degree of polarisation and the position angle for the observations in 1969 and 1972. +, Serkowski 1969; ●, Shawl 1969; ○, our results 1970; □, our results 1971; △, Forbes 1971; ×, Dyck *et al.* 1971; ◇, Serkowski, 1972.

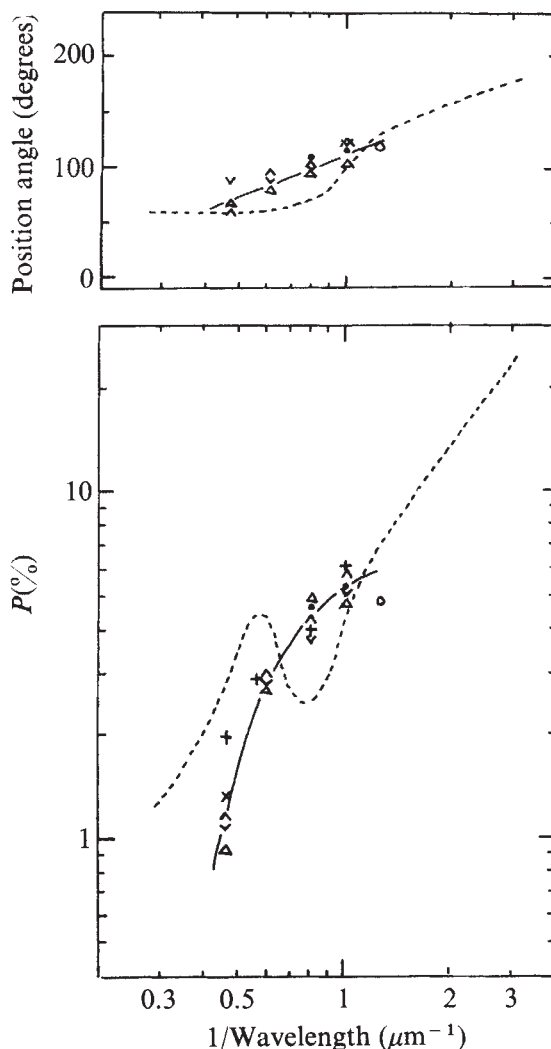
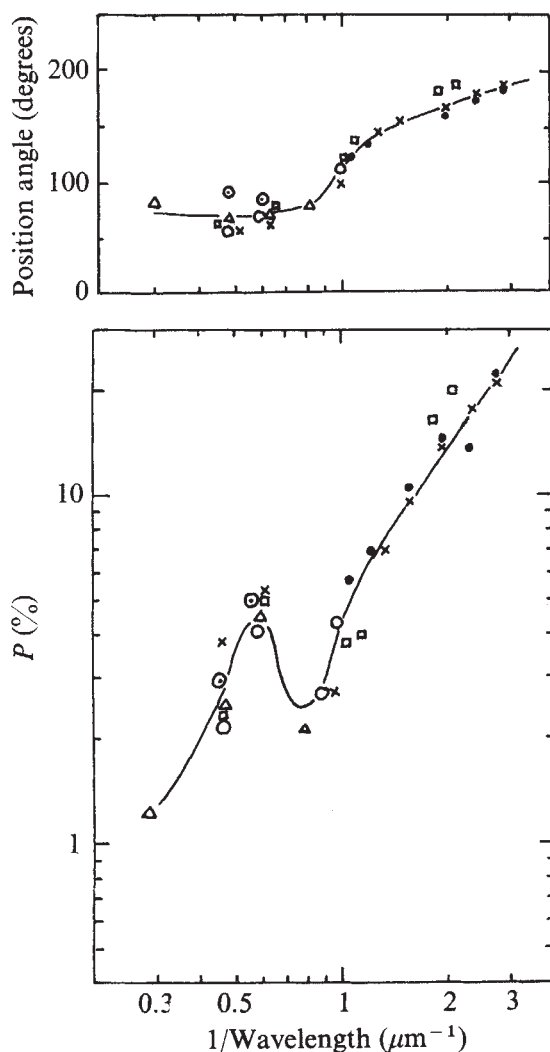


Fig. 2 The same as in Fig. 1 but for 1974 and 1975 (+, January, 1974; ×, ●, △, V, Λ, ○ February–April, 1975).

shown to originate mostly from a reflection nebula⁶. High resolution photographic observations have shown that the nebula is highly polarised ($\leq 70\%$)⁸. Such a high polarisation can be explained only in terms of Rayleigh-like scattering by very small grains of radii $< 0.1\ \mu\text{m}$. These small particles, however, would not be capable of producing the infrared polarisation.

The infrared polarisation would have to be attributed to scattering by much larger grains, of radii $\sim 1\ \mu\text{m}$, concentrated in a fairly dense cloud around the central star. The existence of the polarisation indicates that the distribution of this dust cloud (infrared nebula) is not spherically symmetric, but very asymmetric—probably disk shaped^{5,6}.

We repeated the observation in 1974 and 1975, and found some remarkable changes in the infrared region. The observations were done with the same procedure as before¹, but using a 1-m infrared telescope equipped with a secondary mirror wobbling chopper. The new results are summarised in Table 1 and shown in Fig. 2, in which the average values of the old results are also shown as a dashed line.

Surprisingly, the peak at $1.6\ \mu\text{m}$ has disappeared, the infrared polarisation is smoothly connected with the visible polarisation. Since the observation was regrettably interrupted by the construction of the telescope, when and how the change occurred is not known precisely.

The photometric observations, however, have not shown any significant change in this same period, as shown in

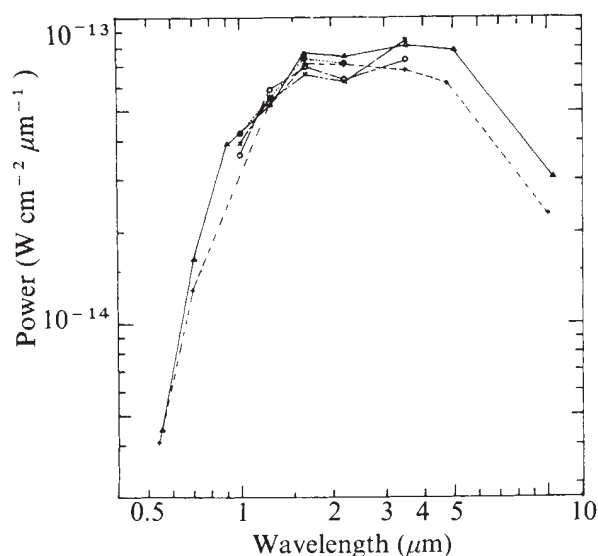


Fig. 3 Photometric observations between 1969 and 1975. (+, Hyland *et al.*, 1969; Δ , Low *et al.* 1970; $\times$, our results, 1971; $\otimes$, our results, 1974; $\odot$, our results, 1975).

Fig. 3. This implies that neither a drastic change in the stellar emission, nor a global change in the configuration of the dust clouds could have occurred.

The change could have been limited to a very localised region with extremely high polarisation. It might have been caused by a dust cloud ejected from the star. An alternative possibility is that the infrared nebula consists of many fragments, and an outer cloud which lets light out through a gap. The motion of the fragments inside the cloud would then bring different fragments to view, through the gap, and the observed polarisation would change. In this case any gross change in appearance would not necessarily be associated with high speed motion of matter. This is more suitable for explaining a change of the polarisation in visible regions^{5,7}, which arises from an extremely large scale reflection nebula.

It has been reported that IRC+10216 showed a periodic variation in infrared polarisation, which correlated with an anti-phase intensity variation⁹. It is interesting in the case of VY Canis Majoris to see whether the change is periodic or not, and what change occurs in optical polarisation as well as in the circular polarisation which had been detected by Serkowski⁵ and Gehrels¹⁰.

Table 1 Polarimetric observations of VY Canis Majoris

Date	$\lambda(\mu\text{m})$	$P(\%)$	Position angle
1974 January 20	1.02	5.7 ± 0.4	—
	1.25	4.0 ± 0.3	—
	1.65	3.0 ± 0.2	—
	2.25	1.9 ± 0.1	—
1975 February 3 February 8 March 1 March 8 March 12 April 9	2.25	1.1 ± 0.1	58 ± 2
	1.02	4.5 ± 0.2	126 ± 1
	1.25	4.0 ± 0.1	108 ± 1
	1.02	4.1 ± 0.4	108 ± 2
	1.25	4.4 ± 0.1	104 ± 1
	1.65	2.2 ± 0.1	87 ± 1
	2.25	0.8 ± 0.2	56 ± 4
	1.02	4.7 ± 0.2	117 ± 1
	1.25	3.9 ± 0.2	108 ± 1
	1.65	2.4 ± 0.1	80 ± 1
	2.25	0.7 ± 0.1	66 ± 1
	1.02	5.4 ± 0.2	118 ± 1
March 12	1.25	4.4 ± 0.2	100 ± 1
	1.65	2.3 ± 0.1	89 ± 2
	2.25	1.1 ± 0.1	53 ± 3
	0.9	5.1 ± 0.1	119 ± 1

For a definite conclusion, much more extensive and continuous observations are necessary in linear and circular polarimetry and photometry from the ultraviolet to infrared regions, as well as photographic studies.

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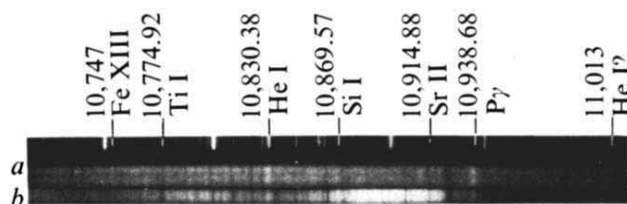
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Fe XIII line in R Aquarii

GREGORY and Seaquist¹ have detected radio emission from the interesting object R Aquarii². In the course of reducing plates of this star made in 1970 and 1971 in the course of my He I, $\lambda = 10,830 \text{ \AA}$ spectral survey, I found the emission line of the forbidden coronal transition of Fe XIII at $\lambda = 10,747 \text{ \AA}$. This line ($^3P_1 - ^3P_0$) is often accompanied by the $^3P_2 - ^3P_1$ line at $\lambda = 10,798 \text{ \AA}$ at high densities ($N_e > 10^9$). The emission line $\lambda = 10,747 \text{ \AA}$ is weak, but clearly seen on two spectra (Fig. 1), with intensity of $\sim 100 \text{ m\AA}$. The spectrum also shows strong emission lines of He I at $10,830 \text{ \AA}$ and $P\gamma$ as well as a line at $11,013 \text{ \AA}$ which may be He I $3'S - 6^1P$. I have examined an old plate made by Merrill in 1948 for traces of the coronal lines at $5,303 \text{ \AA}$ and $6,374 \text{ \AA}$ (Fe XIV and Fe X) and found them absent. G. Wallerstein (personal communication) has kindly examined his plates from 1970–71 and finds the $6,374 \text{ \AA}$ line definitely absent. If the lines are excited by the $2,400\text{-K}$ radiation field of the star, the $6,374 \text{ \AA}$ and $5,303 \text{ \AA}$ lines should be much fainter than the $10,747 \text{ \AA}$, because of their higher excitation energy.

Some idea of the physical conditions in the corona of R Aquarii may be found from the intensity of the line ($100 \text{ m\AA}$) and the absence of other coronal lines. We assume a corona (or part of the nebula) at 10^6 K (coronal ionisation—ionisation at 10^6 K and $N_e < 10^{10}$ —is density independent). If collisional excitation were responsible, we would require $N_e^2 V \sim 3 \times 10^{45} \text{ cm}^{-3}$. In that case the line at $10,798 \text{ \AA}$ would

Fig. 1 Two image tube spectrograms of R Aquarii. *a*, September 1968, phase 0.01 (maximum light); *b*, July 1970, phase 0.79, with identified lines and wavelengths ($\text{\AA}$) marked. The Fe XIII line is at extreme left, near the $10,749 \text{ \AA}$ Si I absorption line. Strong He I and $P\gamma$ emission is also seen. There are a number of other possible emission lines in the spectrum, but none is identified and they may just be windows in the continuum.



be at least as strong as that at $10,747 \text{ \AA}$, however, and the coronal lines in the visible would be excited by the thermal electrons and be much stronger relative to the continuum there. If the coronal density is low, we will still have the ionisation corresponding to the kinetic temperature, but the ions will be excited by photospheric radiation at a rate

$$F_{12} = \frac{1}{w} \frac{g_2}{g} \frac{A_{21}}{\exp(hv/kT) - 1} = \frac{0.05}{w} \text{ s}^{-1}$$

for $T_c = 2,000 \text{ K}$ with w the dilution factor. Integrating this over a uniform nebula, we find

$$R_{\text{cor}} N_{\text{Fe}} = 2.1 \times 10^{14} \text{ cm}^{-2}$$

or

$$R_{\text{cor}} N_e = 2 \times 10^{18} \text{ for } N_e/N_{\text{Fe}} = 10^4$$

The scale height of such a corona, if distributed barometrically, would be 100 AU, but of course the gravity could not keep it from escaping and other factors must play a role. For $\lambda = 5,303 \text{ \AA}$, A_{21} is six times greater, but the exponential term is $10^{5.6}$ greater, so the emission is 6×10^4 less and the line cannot be seen, even though the continuum is lower by 6 mag than the $1.1 \mu\text{m}$ value.

The thermal radio flux from this corona is, at $\lambda = 3.5 \text{ cm}$, $1.44 \times 10^{-16} R_{\text{cor}} \text{ mJy}$. If the source size is $< 1''$, $R_{\text{cor}} < 4 \times 10^{15}$, and a flux of 5.5 mJy is obtained; for $T = 2 \times 10^6 \text{ K}$ (also possible for Fe XIII), we have 8 mJy, in rough agreement with the flux measured by Gregory and Seaquist.

We conclude that R Aquarii is surrounded, *inter alia*, by a corona at $T \simeq 10^6 \text{ K}$ and $R \sim 100 \text{ AU}$, which produces the observed iron line and may be the source of the radio emission observed.

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Note added in proof: A spectrogram obtained on November 23, 1975, on the rise ($M \sim 9$) after an exceptionally deep minimum, showed no Fe XIII emission, but extremely intense P Cygni-type He emission, as well as P γ emission.

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Pulsars and magnetic monopoles

THE report¹ of the possible existence of magnetic monopoles makes some speculations about their astronomical role worthwhile. Pulsars possess magnetic fields, possibly the highest in the Universe², and it thus seems natural to speculate how these magnetic fields might affect monopoles and how the latter, in turn, could influence the behaviour of the pulsars.

For concreteness we adopt Schwinger's dyon picture of magnetic monopoles³. The magnetic charge g is then $6 \times 137 \times e$ and the monopole mass M gives $Mc^2 = 400 \text{ GeV}$. One very important fact is that such a monopole acquires $\sim 0.25 \text{ MeV}$ by travelling 1 cm in a magnetic field of $\sim 1 \text{ gauss}$.

Let us estimate the absorption cross section of a pulsar for a monopole of energy ε . Assuming that the pulsar field is that of a static dipole, we find that the perpendicular momentum acquired, Δp , by a monopole passing at a distance r is given approximately by the relationship (c : velocity of light)

$$\Delta p = g^2 M^2 / \varepsilon r^4 c \quad (1)$$

where $M \simeq B_0 R^3$, is the magnetic moment of the pulsar with maximum surface field B_0 and radius R . Consider a monopole moving along a path which intersects field lines that do not

curve too much along their approach towards the stellar surface (that is, the collision takes place within not too wide a cone around the magnetic axis). This monopole will be appreciably deflected and thus may be channelled into the pulsar polar region, provided $\Delta p \gtrsim p$, or, equivalently, if $r \lesssim \sqrt{(gM/\varepsilon)}$. This leads to a capture cross section σ (up to a numerical factor of less than unity arising from the geometry)

$$\frac{\sigma}{\sigma_{\text{geom}}} \simeq \frac{gM}{\varepsilon R^2} \simeq 3 \times 10^{16} \frac{RB_0}{\varepsilon} \quad (2)$$

where R is the radius in km, ε the energy in MeV, and B_0 is in units of 10^{12} gauss .

It remains to estimate ε . Passage through random magnetic fields in interstellar space probably leads to a substantial acceleration of the monopoles. A further energy gain could be acquired near the pulsar by means of its non-dipolar magnetic field. Such a process would be rather efficient in the vicinity of normal stars where spiralling magnetic field lines carried by the stellar wind extend over large distances: for example, one can estimate that the interplanetary space around the Sun with $B = 10^{-5}$ – 10^{-6} gauss , $l = 10^9 \text{ km}$, would provide $Bl \simeq 10^8$ – 10^9 gauss cm and a pre-acceleration to 10^{14} eV before the actual impact on the dipole field takes place. In contrast, the corresponding mechanism for pulsars is probably much less important, because of the lack of a 'pulsar wind', and because the region where static non-potential magnetic field configurations can occur is limited to within the light cylinder, where Bl is appreciably smaller than in the interplanetary space.

It is probably safe to take an upper limit of $\sim 10^{11} \text{ eV}$ for ε . For the pulsar case, this would make $\sigma/\sigma_{\text{geom}} \sim 10^6$ – 10^7 , making the capture cross section equal to the cross section of the light cylinder, beyond which, anyhow, one would not extrapolate the static formula, or the simple picture of the capture given here. On the other hand for normal stars $B_0 R$ is substantially lower, and $\sigma/\sigma_{\text{geom}} \sim 1$, indicating that in contrast to pulsars, monopoles would arrive at the stellar surface more or less uniformly.

One can thus conclude that pulsars might have a capture cross section for monopoles $\sim 10^{18}$ – 10^{20} cm^2 . Impinging monopoles would be funnelled into the polar regions. Their terminal energy at impact can easily be estimated from

$$\int_{\infty}^R gB(r) dr \simeq gB_0 R$$

to be $\geq 10^{22} \text{ eV}$, if we take $B_0 \sim 10^{12} \text{ gauss}$, $R \sim 10^6 \text{ cm}$.

Along their way to the pulsar surface the monopoles suffer energy losses due to curvature radiation⁴. If this were the only energy loss, their energy balance would be described by the relationship

$$\dot{\varepsilon}/c = Bg - A\varepsilon^4; \quad A = \frac{2}{3}(g^2/\rho^2)(Mc^2)^{-4} \quad (3)$$

where ρ is the radius of curvature of the trajectory. The resulting saturation energy ε_{max} is given by the equation

$$\varepsilon_{\text{max}}/Mc^2 \simeq (B\rho^2/g)^{1/4} \quad (4)$$

which leads to $\varepsilon_{\text{max}} \simeq 10^{21}$ – 10^{22} eV (probably an underestimate) if we take for ρ the radius of curvature of the last open field line, or $\rho \simeq$ radius of the light cylinder $\simeq 10^8$ – 10^{10} cm . The value of ε_{max} found here is sufficiently large that the value of the terminal energy we found earlier is not appreciably affected. We note, by the way, that the ratio of the radiation reaction force, $2g^4 B^2/3M^2 c^4$, to the accelerating force Bg is $10^{-61} B$ (gauss), which is so small that the ordinary radiation damping can be neglected.

Although a monopole crossing the pulsar magnetosphere will also be affected by the electric field in it⁴, its gyroradius r in

an electric field E , which is given by the equation

$$r = \varepsilon/gE \quad (5)$$

will be sufficiently large not to affect appreciably the monopole trajectory.

Let us now investigate whether the pulsar magnetic field could be substantially extinguished by the accretion of monopoles. For this to happen, each pulsar hemisphere has to collect N monopoles such that $NgR \simeq B_0 R^2$. The number of required monopoles is then

$$N \simeq B_0 R^2/g \simeq 2 \times 10^{30} \quad (6)$$

If we take the pulsar lifetime to be $\sim 10^{15}$ s, the cosmic flux of monopoles must be $\lesssim 10^{-4} \text{ s}^{-1} \text{ cm}^{-2}$, as the extinction of the pulsar magnetic fields seems not to have taken place. This upper limit is 6 orders of magnitude higher than that set already by similar considerations about the Earth's magnetic field^{5,6}, and 9 orders of magnitude higher than that set by the lack of more than one event found in cosmic ray work¹.

It thus seems that no useful conclusions regarding the flux of cosmic magnetic monopoles can be drawn from the persistence of pulsar magnetic fields. The situation is not, however, quite as simple as this, because of the high terminal energy of the monopoles. In fact, near the pulsar surface, the accelerating field imparts to the monopole an energy equal to the monopole rest mass energy over a distance of $< 10^{-6}$ cm. In a collision with a nucleus, there will be enough energy in the centre-of-mass system to produce a pair of monopoles, if the γ of the monopole equals or exceeds $\gamma_0 = 2M/m_p \simeq 1,000$ (m_p : proton mass), or $\varepsilon \simeq 10^{15}$ eV. We see that there will thus be ample energy to create monopole pairs. The wrongly charged member of the pair will be hurled back into extra-pulsar space with an energy $> 10^{20}$ eV (compare the mechanisms for pair production in the Sturrock⁷ and Ruderman-Sutherland⁴ theories of pulsar emission) while its companion will be further accelerated towards the pulsar. If the conditions

$$\gamma_0 Mc^2/gB < \lambda_{\text{loss}} \text{ and } \lambda_{\text{pair}} \ll l^* \quad (7)$$

are satisfied, where λ_{loss} is the mean free path for energy losses not associated with pair creation, λ_{pair} is the pair creation cross section mean free path and l^* the width of the region with a density sufficiently high for copious pair creation to occur, an avalanche of new monopoles may be generated. The energy loss of a monopole in ordinary matter has been estimated⁸ to be $10^{12} \text{ eV g}^{-1} \text{ cm}^2$. A layer of width $\sim 10^{-1}$ cm in the outer crust with a density $\sim 10^4 \text{ g cm}^{-3}$ would be a suitable environment for the avalanche production of monopoles. If we estimate the number of monopoles N_* produced in the avalanche to satisfy the relationship

$$N_* Mc^2 \gamma_0 = \varepsilon \quad (8)$$

and use $\varepsilon \simeq 10^{21}$ eV, we find $N_* \simeq 10^6$, and the persistence of the pulsar magnetic fields would lead, for pulsars such as PSR 1933 or PSR0527 with a relatively short lifetime (and a large B_0 in the case of PSR0527), to quite a low upper limit on the monopole flux, which may even be as stringent as the one given in ref. 1.

It is, of course, impossible to estimate N_* in any reliable way, as long as data on monopole pair-production cross sections are lacking. Nevertheless, in view of the availability in the pulsar crust of a relatively large region where the monopoles undergo frequent collisions while still, on the average, gaining energy from the magnetic field, a very substantial multiplication can be expected to occur.

To summarise our main conclusions: first, the persistence of pulsar magnetic fields may possibly set a more stringent limit on the cosmic flux of magnetic monopoles than the presently available one. The actual value of the limit can only be given,

once the pair-production cross section is known. Second, pulsars may act as copious sources of very high energy (secondary) monopoles (if monopoles really exist). It would be of interest to look for correlations between monopole events and pulsar positions, if monopole events are observed in any significant number.

The annihilation of a pair of oppositely charged monopoles will surely have an extremely low probability. Even if there were the 10^{30} monopoles needed to extinguish the pulsar magnetic field, their density would only be $\sim 10^{12} \text{ cm}^{-3}$, and the annihilation mean free path would be many orders of magnitude larger than the pulsar radius. If the monopoles are also electrically charged, as in the dyon model³, their accumulation would induce an additional electric dipole field which would, however, be $\lesssim 10^7 \text{ V cm}^{-1}$ so that its effect could be negligible compared to the already existing magnetospheric field.

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No anisotropy in angular diameter-redshift relationship

USING data for the brightest cluster galaxies, we here consider whether any anisotropy exists in the angular diameter-redshift relationship. This is in response to the finding of Rubin *et al.*¹, who described a 'curious' distribution of radial velocities for Scl type galaxies having apparent magnitudes m in the range $14 < m < 15$. For two distinct regions of sky, the mean radial velocity for these galaxies differs by $\sim 1,400 \text{ km s}^{-1}$; the corresponding mean apparent magnitude differs by ~ 0 mag, compared with the difference of 0.5 mag expected on the basis of this velocity difference. They tentatively concluded that the Hubble parameter H is not isotropic.

Le Denmat and Vigier² examined the distribution of HM (see ref. 1) for type I supernovae³ that have occurred in a wide variety of galaxies, and found a highly significant anisotropy that agrees with that described by Rubin *et al.* We note, however, that the supernova apparent magnitudes given in ref. 3, and used by Le Denmat and Vigier², seem to be mostly post-maximum values and therefore only set lower limits on HM : little weight can therefore be placed on this result. Jaakkola *et al.*⁴ have also found an anisotropy in HM for compact galaxies with absorption spectra, but they further found that the degree of anisotropy decreases with increasing distance.

If the effect found by Rubin *et al.*¹ is truly cosmological in origin, and is not the result of selection effects, then it should manifest itself in other 'cosmological' relationships, such as the angular diameter-redshift relationship. Rubin *et al.*¹ did not find, with certainty, any difference in the mean angular diameter of the Scl galaxies in their sample, but they described the values they used as being of low weight.

Data obtained⁵ from measurement of 48'' Schmidt plates form a reasonably homogeneous sample of isophotal angular

diameters for E galaxies having redshift z in the range 0.004–0.085. Less extensive and less homogeneous are the data measured from 200'' plates, also tabulated by Sandage⁵. We neglect both the 'K-like' correction, that transforms angular diameters to the rest frame of each galaxy (this correction amounts to $\sim 6\%$ at most for the 48'' data), and the 'seeing' correction ($\lesssim 1''$), which is small by comparison with the smallest angular diameter in the 48'' sample ($\sim 10''$). For the smallest angular diameters in the 200'' sample, the net effect of these two corrections is zero⁵.

Isophotal angular diameters, θ , are proportional to z^{-1} for $q_0 = +1$, which is a sufficiently good approximation for our range of z values. For each galaxy we compute the quantity $A = \log \theta + \log cz$, which is analogous to the HM of Rubin *et al.*¹. A , which for low redshifts is a measure of the linear dimensions of galaxies for uniform H , is not correlated with z for either sample. Writing $\langle A \rangle$ for the mean value of A in each region, we should expect

$$\langle A \rangle_{II} - \langle A \rangle_I = \langle HM \rangle_{II} - \langle HM \rangle_I = \sim 0.1$$

(see ref. 1) if the anisotropy¹ is indeed 'cosmological'. If the alleged anisotropy is not, however, cosmological then we should expect that the difference in the $\langle A \rangle$ should merely reflect the sampling error, that is $\langle A \rangle_{II} - \langle A \rangle_I = 0$.

For the 48'' sample, $\langle A \rangle_{II} - \langle A \rangle_I = 0.024 \pm 0.030$, a difference that is not statistically significant (probability $P = 0.44$). In addition there is no tendency for high or low values of A to be correlated with the regions I and II of Rubin *et al.*¹ ($P = 0.52$). Even if we suppose that the above difference in $\langle A \rangle$ arises entirely from a difference in H (that is, we assume that the brightest cluster galaxies in the 48'' sample are both standard candles and standard rods) then $H_{II}/H_I = 0.95^{+0.55}_{-0.35}$ from the isophotal angular diameter-redshift relationship. This ratio is not significantly different from unity, and in any case is in the opposite sense to that claimed previously^{1,2,4}. We note also that no significant difference between regions I and II is found when metric, rather than isophotal, angular diameters are considered. Furthermore, no anisotropy exists in regions of sky other than those delineated by Rubin *et al.*¹, nor does anisotropy become apparent when various redshift intervals are considered.

Unfortunately sufficiently accurate and homogeneous apparent magnitude values were not available for the 48'' sample so we could not test whether the $\langle HM \rangle$ for this sample differs significantly between regions I and II. For the smaller and less homogeneous 200'' sample, however, (for which $\langle A \rangle_{II} - \langle A \rangle_I = 0.051 \pm 0.037$; $P = 0.20$), $\langle HM \rangle_{II} - \langle HM \rangle_I = 0.034 \pm 0.050$ which does not differ significantly from zero ($P = 0.50$). It should be noted, however, that Jaakkola *et al.*⁴ have found that the anisotropy is apparently present in their sample of brightest cluster galaxies.

If the anisotropy apparent in the HM values of ScI galaxies and compact galaxies with absorption spectra is indeed cosmological, then it could be masked in the angular diameter-redshift relationship by the intrinsic dispersion in the linear dimensions of E galaxies. After removal of dispersion from measuring errors, Sandage⁵ estimates that the linear diameters of brightest cluster galaxies have a dispersion of $\sim +20\%$; the corresponding dispersion in $\log \theta$ is ≈ 0.1 . Neglecting errors in redshift values, the dispersion in A arising from this dispersion is ~ 0.1 , which is precisely the magnitude of the difference $\langle A \rangle_{II} - \langle A \rangle_I$ expected on the basis of the difference $\langle HM \rangle_{II} - \langle HM \rangle_I$.

On the other hand, it has similarly been argued⁶ that the dispersion in the absolute magnitudes of the galaxies considered could, in certain circumstances, give rise to an apparent anisotropy in HM . Although Rubin *et al.*¹ regarded the possibility of large scale inhomogeneities unlikely as a means of accounting for the anisotropy, Sandage and Tammann⁶ have suggested that inhomogeneities on a scale ~ 100 Mpc compounded with the narrow range of m values considered in ref. 1,

could explain the Rubin *et al.* result. The equality of the $\langle m \rangle$ values for regions I and II would then merely reflect the narrowness of the apparent magnitude range chosen by comparison with the dispersion in the absolute magnitudes of ScI galaxies (0.4 mag, ref. 7). Jaakkola *et al.*⁴, however, found that the anisotropy does seem to exist ($P = 0.015$) for compact absorption galaxies with $13 < m < 18$.

A further consideration in discussions of effects of this kind should, however, be the homogeneity of the data used. Sandage⁸ has argued that much of the dispersion in the absolute magnitudes of brightest cluster galaxies arises from differing observational errors (that is, different observing sites and observing conditions) and clearly this argument would also apply to the absolute magnitude dispersions of other types of galaxy. Similarly, the effect of personal observing habits, as discussed by Penston and Rowan-Robinson⁹ in connection with the alleged anisotropic redshift distribution of quasars, could also give rise to systematic observational errors.

Data used in previous examinations^{1,2,4} of the Hubble anisotropy have been taken from catalogues in which apparent magnitude values have been obtained from a number of sources and it would be difficult to argue that these data are sufficiently homogeneous in the sense discussed above. Thus any anisotropy in the apparent magnitude-redshift relationship should be regarded as 'not proven' until analysis of a sufficiently homogeneous sample of galaxies shows otherwise.

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Present trends in the Earth's magnetic field

WE outline here some of the implications of the latest in a series of models of the Earth's magnetic field derived in association with the World Magnetic Charts published by the British Hydrographic Office. The model, details of which are published elsewhere¹, is defined by spherical harmonic coefficients representing the internal part of the magnetic field (to twelfth order and degree; 168 coefficients), its secular variation (to eighth order and degree; 80 coefficients), and its secular acceleration (the 26 most significant coefficients) at epoch 1975.0. Because of the greatly improved distribution, accuracy and quantity of data incorporated in the analysis, we believe that this model, particularly with regard to the secular variation, provides a significantly better representation of the Earth's magnetic field than any previous model. It is of interest to compare some of the parameters derived from this model with the trends shown by its predecessors.

To a first approximation (and an extremely good one) the Earth's magnetic field can be represented by that of a uniformly magnetised sphere—the so-called 'centred dipole' model. The orientation of the dipole axis (78.7°N, 289.5°E) and its motion

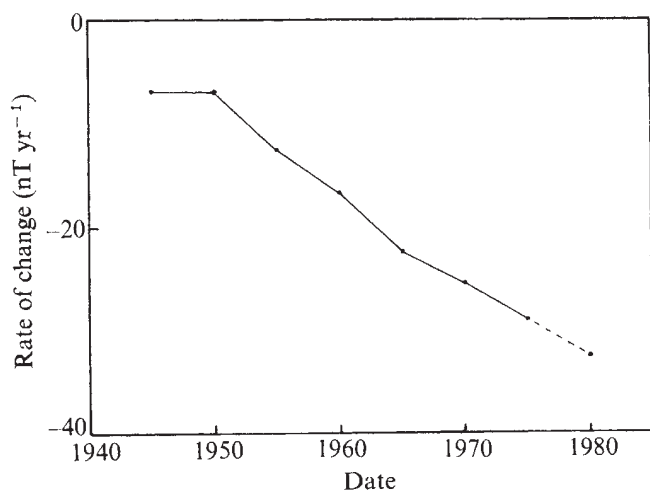


Fig. 1 Rate of decrease of dipole moment. The point for 1970 is from an unpublished model based on observatory data; that for 1980 is obtained by updating the 1975 model using its time-dependent coefficients, and is included to show the trend they imply.

($0.00^\circ \text{ yr}^{-1} \text{ N}$, $-0.06^\circ \text{ yr}^{-1} \text{ E}$) follow closely the trends found by Malin³, although they suggest that the westward motion of the axis is slowing down. It is usual to measure the strength of the dipole by MR^{-3} , where M denotes the dipole moment and R the mean radius of the Earth. For the present model, this quantity is 30,701 nT, and is decreasing by 28.8 nT yr^{-1} . This rate of decrease is the largest yet found, but is in good agreement with the rather speculative trend noted earlier (see refs 3 and 4, and Fig. 1). The recent points in Fig. 1 are in excellent agreement with the predictions of Malin and Clark⁴ which were based on the assumption of a linear change in the decrease of dipole moment, and there seems little reason to revise their estimate that, if present trends were to continue, the field would reverse in about 2230 AD.

A better approximation to the main field is obtained by displacing the dipole from the Earth's centre, without changing its magnitude and direction, to the magnetic centre—the 'eccentric dipole' model. The displacement vector is (474 km, 19.8° N , 148.0° E) and its rate of change (2.2 km yr^{-1} , $0.26^\circ \text{ yr}^{-1} \text{ N}$, $-0.05^\circ \text{ yr}^{-1} \text{ E}$). The distance of the magnetic centre from the centre of the Earth continues to increase at a remarkably constant rate; there is no support to the suggestion⁵ that

the magnetic centre is moving in an ellipse. The rate of northward drift continues to increase slowly, but, more interestingly, the westward drift, previously seen to be slowing down⁴, has now virtually stopped.

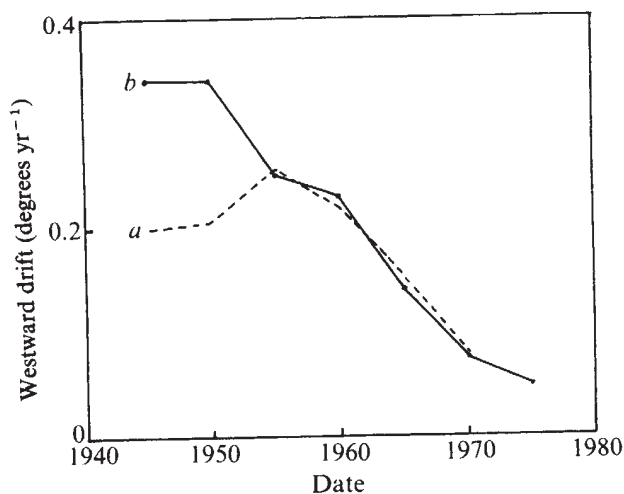
The magnetic dip poles are those points on the Earth's surface at which the lines of force are vertical. The model indicates that the north magnetic dip pole is located on Bathurst Island at (76.1° N , 259.5° E) moving eastwards at 5 km yr^{-1} . The south pole is at (65.6° S , 139.6° E), about 120 km off the coast of Adelie Land, and is moving approximately north-eastwards, towards New Zealand, at 5.5 km yr^{-1} . The exact positions and movements of such features as the dip poles may be significantly affected by nearby anomalies, and are better determined by local surveys than by a global model. The positions and movements are, however, entirely consistent with the survey data obtained near the north magnetic pole⁶. Recent survey data are not available for the vicinity of the south magnetic pole, but the declination measures from the nearby observatory of Dumont d'Urville are in good agreement with those expected to be associated with the pole. It is interesting to note that both of the dip poles show an eastward rather than a westward motion.

The evidence seems to indicate that the westward drift of the magnetic field, which has been going on at least since the time of Halley⁷, has recently slowed down and may even have stopped. A numerical estimate of the westward drift is obtained by taking a global mean of longitudinal movements of certain features of the Earth's magnetic field. The best method of taking this mean, and the most appropriate features to consider are still open to discussion. For example, should one consider total intensity, B , or vertical intensity, Z ; pattern at the surface of the Earth or at the core-mantle boundary; the whole field or only the non-dipole part of the field? The results obtained from several of these methods are given in Table 1.

Table 1 Westward drift (degrees yr^{-1})

	1975 (ref. 1)	1942.5–1962.5 (ref. 2)
Centred dipole	0.06	0.09
Eccentric dipole	0.05	0.29
Richmond's method (ref. 8):		
Whole field (Z)	0.09	0.13
Whole field (B)	0.10	0.15
Non-dipole field (Z)	0.13	0.21
Non-dipole field (B)	0.12	0.17
Yukutake's method (ref. 9):		
Whole field	0.07	0.14
Non-dipole field	0.10	0.24

Fig. 2 Westward drift in degrees yr^{-1} . *a*, From length of day data; *b*, for the eccentric dipole. Point for 1970 *b* as in Fig. 1.



Although the different estimates vary considerably, they all agree that the westward drift is much smaller in 1975 than it was during the interval 1942.5–1962.5, by a factor of two on average. The main reason for this is the diminution in the secular variation of the h_2^1 term, which has for many years been the major contributor to westward drift, and accounted for the large apparent difference between the drift rates of the dipole and non-dipole parts of the field noted in the past. A more reasonable division might have been into h_2^1 and non- h_2^1 parts of the field. Traditionally, the westward drift has been interpreted as the difference between the rotation rates of the outer layers of the core (where the field originates) and the mantle. If that were so, the recent reduction in westward drift would imply either a transfer of angular momentum from the mantle to the core—which should be accompanied by an increase in the length of the day—or a redistribution of angular momentum within the core. Following Vestine and Kahle¹⁰ we have adopted the former interpretation, calculated the westward drift that would account for the observed length of day (Fig. 2*a*), and compared this with the observed westward drift of the eccentric dipole (ref. 4 and Fig. 2*b*). The mean ordinate of Fig. 2*a* is arbitrary, and

its scale can be adjusted by altering the thickness of the layer of outer core to which angular momentum is transferred. We have chosen values that bring the recent portions of curves *a* and *b* close together, but to achieve that the thickness of the core layer had to be reduced to the improbably low value of 100 km. Even so, there seems to be little correlation between the two curves, and we conclude that a direct relationship between the westward drift and the length of day has yet to be established.

Regardless of the physical interpretation of westward drift, it provides a simple concept that accounts for much of the secular change of the non-dipole part of the field (though its deficiencies in this respect have been emphasised by James¹¹). A better approximation to the secular change is given by a simple rotation of the non-dipole part of the Earth's magnetic field about an axis other than the geographical axis¹². For the 1975 model, the rotation rate is $0.145^\circ \text{ yr}^{-1}$ in a clockwise direction (when viewed from above) about an axis with its pole at $(76.1^\circ \text{N}, 273.0^\circ \text{E})$. The close proximity of this pole of rotation to the dip pole is attributable to chance, as may be seen by examining earlier positions for the pole of rotation¹³. This latest result confirms and extends the trends discussed by Malin and Saunders¹³.

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Possible late Precambrian subduction zone in South West Africa

CONTINENTAL margin plate tectonism during the late Precambrian in southern and central South West Africa and western Botswana is strongly suggested by the geographical distribution, stratigraphy, composition and evolutionary characteristics of the pre-Damara, pre-Nama formations (1,350–900 Myr old) occurring north of the Namaqua Tectonic Province¹. If the distribution of these units is considered it is apparent that a unique pattern exists (Fig. 1). The data for the generalised geological map of Fig. 1 are taken from various sources^{2–9} and show: first, the distribution has the form of a prominent and extensive curvilinear feature or 'arc'. Second, the arc separates the undeformed late Precambrian deposits (Nama Group¹⁰) of the stable platform area—the Kalahari Craton or Plate—from the highly deformed and metamorphosed deposits of the late Precambrian Damara orogenic belt, suggesting that the arc occupies a marginal cratonic position, and third, the trend of the arc and arc structures (such as major faulting) parallels that of the Damaran structural trends, suggesting a possible relationship between the geotectonic development of the arc and the Damaran Orogeny (see Fig. 1).

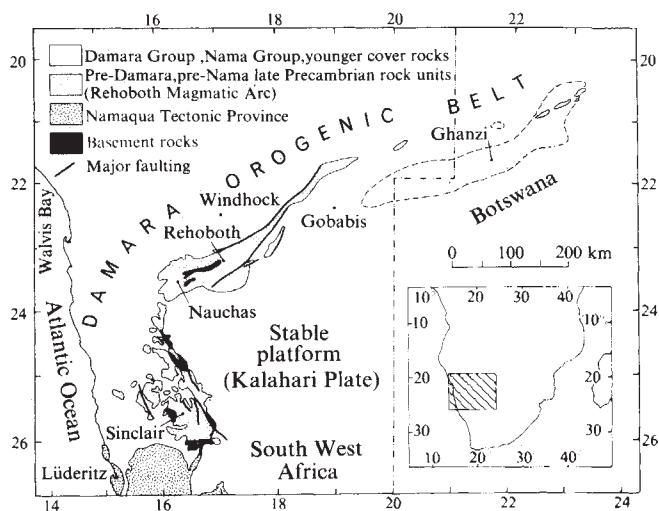


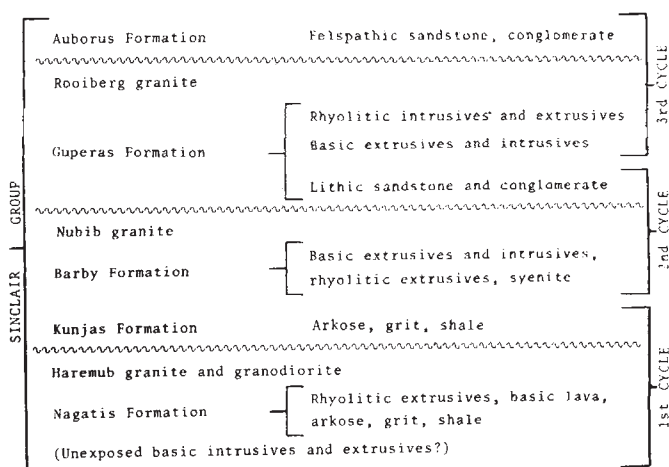
Fig. 1 Distribution of the pre-Damara and pre-Nama late Precambrian formations in southern and central South West Africa and western Botswana.

The various units comprising this late Precambrian arc consist essentially of highly variable sequences of basic, intermediate and felsic lava and volcanics, as well as thick sedimentary deposits which are frequently of obvious local provenance. The sequences were extruded on to and deposited on an older sialic basement of unknown age and were intruded by large amounts of comagmatic high level granitic, rhyolitic and basic to intermediate material. The name Rehoboth Magmatic Arc has been proposed for this volcano-plutonic belt⁹.

The best exposed and most intensively studied part of the belt is the southern portion which is the type area for the Sinclair Group⁹, the geological succession of which is summarised in Fig. 2. The evolution of the Sinclair Group proceeded within three major cycles. The basis for this cyclic subdivision is seen in a repeated pattern of development. The generation and emplacement of basic to intermediate magma into and on to the continental crust, followed by the generation and emplacement of felsic magma into high crustal levels, subsequent vertical tectonics and production of local fault trough basins, followed by erosion of the earlier igneous units and rapid deposition of immature clastic debris into the local basins. The cycle ends with a period of relatively little faulting and tilting.

Basic and intermediate volcanism is shown by extensive piles of calc-alkaline and, to a lesser extent, tholeiitic,

Fig. 2 Geological succession and evolutionary cycles of the late Precambrian Sinclair Group, South West Africa.



flows, building up locally to thicknesses of 8,000 m within individual cycles. The calc-alkaline lavas are characteristically highly porphyritic (plagioclase feldspar and/or pyroxene) and range from 'normal' basalts, basaltic-andesites and andesites to highly potassic varieties having all the compositional and modal characteristics of shoshonites^{11,12}. Basic intrusives are comagmatic and represented by tholeiitic gabbros often displaying igneous layering, by basaltic dykes and by bodies of calc-alkaline porphyritic basalt, gabbro, diorite, monzonite and syenite.

Felsic units are high level granite plutons and batholiths, rhyolitic dykes, plugs, domes, flows and volcanoclastics. A distinct compositional break exists between the felsic and basic igneous rocks of the Sinclair Group, and the available evidence indicates that the felsic magmas represented the first products of melting of predominantly lower crustal material⁹. Heat for the fusion process was evidently supplied by the enormous volumes of basic magma transported through the crust, resulting in the basic, followed by felsic, succession, of magmatic events found in the various evolutionary cycles of the group. The apparent lack of an extensive basic magmatic event initiating the first cycle could conceivably be attributable to emplacement of magma into a relatively cool crust and consequent rapid and early chilling, with only minor volumes of basic magma reaching the surface. Furthermore, the emplacement and deposition of the younger units has largely obliterated evidence for the early stages of the first cycle.

Cessation of magmatic activity within each cycle was followed by periods of intense erosion of local volcanic piles and exposed plutons with the deposition of immature clastic debris into local basins which began forming toward the latter stages of the felsic magmatic events. The basins were the result of vertical movement along extensive faults paralleling the general trend of the Rehoboth Magmatic Arc, which in the Sinclair Group type area, is approximately north-north-west.

Other parts of the arc provide evidence for similar evolutionary patterns to that of the Sinclair Group^{3,4,6-8} although never as complete or as well preserved; the ENE trending sector has been involved to varying degrees in the younger Damaran tectonism, with thrusting and shearing to the SSE. Sequences are typically of limited lateral extent with interfingering relationships, consisting of thick piles of basic, intermediate and felsic volcanics, and immature clastics; comagmatic intrusion is extensive. Granodiorite and quartz diorite (as opposed to highly siliceous granites) are, however, more common within the Nauchas-Rehoboth sector of the arc than in the Sinclair Group type area.

I regard the characteristics of the Rehoboth Magmatic Arc and its constituent units as reasonable evidence for the presence of a subduction zone active during late Precambrian times beneath the western and north-north-western margin of the Kalahari Plate. In terms of modern plate tectonics a requisite for continental margin tectonics is the presence of a stable plate, and the Kalahari Plate has certainly remained essentially undeformed since Sinclair Group times (since ~ 1,350 Myr), inferring that the craton had acquired its present stable and consolidated nature before the development of the Rehoboth Magmatic Arc.

The well-defined curvilinear shape, situated at the edge of a highly stable continental plate, thick piles of tholeiitic, calc-alkaline and shoshonitic extrusives and comagmatic intrusives, voluminous 'granite' batholiths and rhyolitic lava, intrusives and volcanoclastics, episodic or pulsing development, vertical tectonics paralleling the trend of the arc, and rapidly deposited immature clastic debris of local provenance, are all characteristics of the Rehoboth Magmatic Arc that are common to young continental margin plate tectonic regimes^{13,16}.

It is suggested, therefore, that the presence of this volcano-plutonic arc can be used to define roughly the position

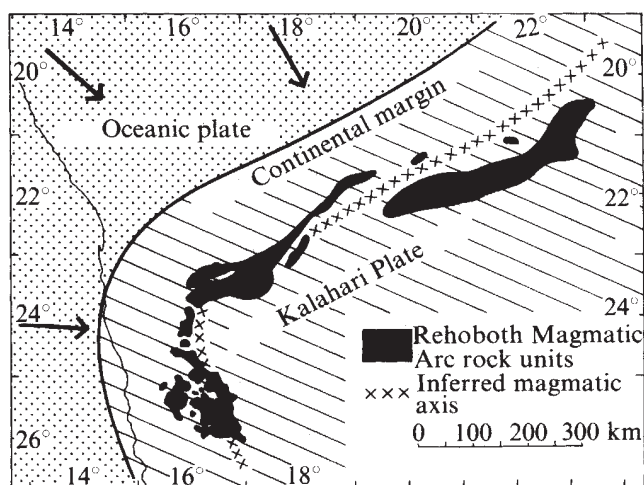


Fig. 3 Suggested reconstruction of the geotectonic setting that may have prevailed during the evolution of the late Precambrian Rehoboth Magmatic Arc, superimposed on a sketch map showing the present-day distribution of the Arc units. Arrows indicate the approximate directions of plate movement.

of the margin of the Kalahari Plate during immediately pre-Damara times. The distance between the continental margin and the axis of a volcano-plutonic arc developed above a subduction zone at a continental margin has been estimated to be in the order of 225 ± 50 km (ref. 17). To the west of the Sinclair area (the southern sector of the Rehoboth Magmatic Arc (Fig. 1)) younger Damara rocks are encountered 180 km to the west along the present coastal regions of South West Africa. It is probable, therefore, that these younger rocks delineate fairly accurately the position of the continental slope regions of the Kalahari Plate during the late Precambrian. In the east-north-east trending sector of the arc, thrusting of the Damara Group units (during the Damaran Orogeny) to the south-south-east over the magmatic arc units has occurred and the original position of the continental margin is a matter of speculation. A suggested reconstruction of the Kalahari Plate margin and prevalent geotectonic setting during evolution of the late Precambrian Rehoboth Magmatic Arc is presented in Fig. 3.

The model proposed here for the evolution of the Rehoboth Magmatic Arc may have far-reaching implications for the development of the Damara Belt, because it implies an oceanic crust during pre-Damara times in the area that is now overlain by the Damara Group. Oceanic crust in the area during the late Precambrian would be consistent with a paratectonic or collision origin for the Damara, as suggested for all pan-African orogenic belts by Burke and Dewey¹⁸. Such a model can, therefore, reconcile the apparently conflicting evidence for the origin of the Rehoboth Magmatic Arc by continental margin subduction, with the supposed ensialic character¹⁹ of the following Damaran orogenic event.

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A novel beryllsilicate phase containing 3-coordinate beryllium ($\text{Rb}_2\text{Be}_2\text{Si}_2\text{O}_7$)

BERYLLSILICATES are normally framework structures, with Be in tetrahedral coordination, similar to Si (ref. 1), and the structural role of Be can be compared with that of Al in the aluminosilicates. We report here on the preparation and X-ray crystal structure determination of $\text{Rb}_2\text{Be}_2\text{Si}_2\text{O}_7$, which contains planar BeO_3 groups. Such coordination is unusual for beryllium, and, in complex oxides, has apparently been found previously only in Y_2BeO_4 (ref. 2), $\text{Ca}_{12}\text{Be}_{17}\text{O}_{29}$ (ref. 3), and K_2BeO_2 (ref. 4).

The compound $\text{Rb}_2\text{Be}_2\text{Si}_2\text{O}_7$ was prepared by solid-state reaction between appropriate quantities of Rb_2CO_3 , BeO and quartz. A single crystal was selected from a sample which had been heated at 950 °C for three days. The orthorhombic cell dimensions are

$$a = 8.92 \text{ \AA}; b = 8.32 \text{ \AA}; c = 5.15 \text{ \AA}$$

The intensity data, obtained from a Hilger and Watts Y-190 linear diffractometer with $\text{MoK}\alpha$ radiation, indicated a body-centred space group. The solution of the vector (Patterson) map indicated the space group to be $\text{I}2\text{mm}$. Later, a few, rather weak, additional reflections were observed on films, and the space group $\text{P}2\text{nn}$ was adopted. The complete solution of the structure using vector and Fourier methods, followed by least squares refinement, has so far given values of $R = 0.071$, over 241 independent reflections.

The structure consists of Si_2O_7 groups, linked at their corners by Be, to form an infinite framework anion of empirical formula $[\text{Be}_2\text{Si}_2\text{O}_7]^{2-}$. Large cavities contain two crystallographically distinct Rb^+ ions, which have 13 and 14 nearest neighbour oxygen atoms (within 3.8 Å), respectively. In the planar BeO_3 group, Be–O distances are around 1.54 Å, which is about 0.11 Å shorter than those in tetrahedral BeO_4 (ref. 5); O–Be–O angles are close to 120°.

X-ray powder diffraction data suggest that $\text{Cs}_2\text{Be}_2\text{Si}_2\text{O}_7$ is isostructural with $\text{Rb}_2\text{Be}_2\text{Si}_2\text{O}_7$.

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Coccolith blooms in the Kimmeridge Clay and origin of North Sea Oil

In this paper I suggest that Kimmeridge Clay oil shales (and therefore much of the oil in the North Sea) were formed from algal blooms, in an environment between open ocean, and an enclosed marine basin. These conditions favour the production of such blooms, which by deoxygenating and poisoning the water, could temporarily create bottom conditions suitable for the formation of organic-rich sediment.

Thin coccolith-rich bands were first noted in the Kimmeridge Clay by Downie¹, at four levels in the cliff sections near Kimmeridge Bay, Dorset. The most prominent of these bands, the White Stone Band, was subsequently recorded in boreholes in Hampshire, Surrey, Sussex² and Norfolk³.

Coccolith-rich bands of a similar nature have recently been recorded at three levels in a borehole at Donington on Bain, Lincolnshire, each level corresponding with one of the higher levels in Dorset. Re-examination of borehole material from Surrey and Norfolk has shown that the same three horizons are present in Surrey, and that the lowest two of these are present in Norfolk (Fig. 1). Thus, these coccolith-rich bands occur over an area where the Kimmeridge Clay varies in thickness from < 100 m to > 500 m, and which embraced both the stable margins and the more rapidly subsiding parts of the basin of deposition.

The coccolith-rich bands are readily recognisable in borehole cores by their pale colour and lightness in weight, and at outcrop by their almost white weathering patina. In Dorset the thickest band is ~ 0.5 m thick, but elsewhere the three bands are each generally < 0.1 m thick. The coccolith contents of these bands ranges from 60–98% of the calcium carbonate fraction of the rock (30–> 50% of the whole rock). Most other Kimmeridge Clay lithologies contain < 3% coccoliths/whole rock, although some of the more calcareous clays have yielded up to 12.5% coccoliths/whole rock.

The coccolith-rich bands are generally composed almost wholly of one species, *Ellipsagelosphaera britannica* (Stradner)³. Even now, seasonal blooms of coccoliths consisting largely of one species occur from time to time over large areas of the North Sea⁴. Such blooms characteristically form in seas which are rich in land-derived nutrients and are to some degree land-locked so that, although they remain a fully marine environment, their salinities are slightly lower than those of the open oceans. The persistence and composition of the thin coccolith-rich beds in the Kimmeridge Clay suggests that they were formed from similar blooms.

In addition to their usefulness as stratigraphical marker beds the coccolith-rich bands may provide an insight into the conditions of depositional environment in which the Kimmeridge Clay oil shales were deposited, since each of the coccolith-rich bands recorded to date occurs in close association (usually interlaminated) with oil shales.

Although they vary considerably in thickness, the Kimmeridge Clay sequences in Dorset, Surrey and Norfolk can be matched with one another in lithological and faunal detail³, suggesting that ecologically similar environments existed over much of southern England, in spite of great variations in rate of subsidence of the sea floor. The distribution of the Kimmeridge Clay lithologies and fauna, mostly pelagic ammonites and benthonic bivalves, including oysters, preserved in calcite, suggests accumulation in a relatively shallow marine environment on a broad continental shelf. The oil shales contain a richer and more abundant fauna than any other lithology in the Kimmeridge Clay. They are characterised by bedding planes crowded with one or two species of small bivalve or ammonite preserved in either calcite or pyrite, and by small gastropods, phosphatised fish debris and faecal pellets, abundant Foraminifera and, locally, by pyritised plates of the free-swimming crinoid *Saccocoma*.

In Dorset the oil shales contain ≤ 40% of brown organic matter which Forsman⁵ has described as composed mostly of kerogen. This kerogen occurs largely as laths of amorphous organic matter (AOM) but is rich in palynomorphs such as dinoflagellates¹. Cosgrove⁶ recorded abnormally high iodine and bromine contents in some of the oil shales from the Kimmeridge Clay in Dorset, and has related this to the abundance of microplankton present.

According to Trask⁷, few modern typically marine sediments contain more than 10% of organic matter; near-shore sediments generally contain between 1% and 8% and open-ocean sediments ~ 1%. Kaplan and Rittenberg⁸ state that the

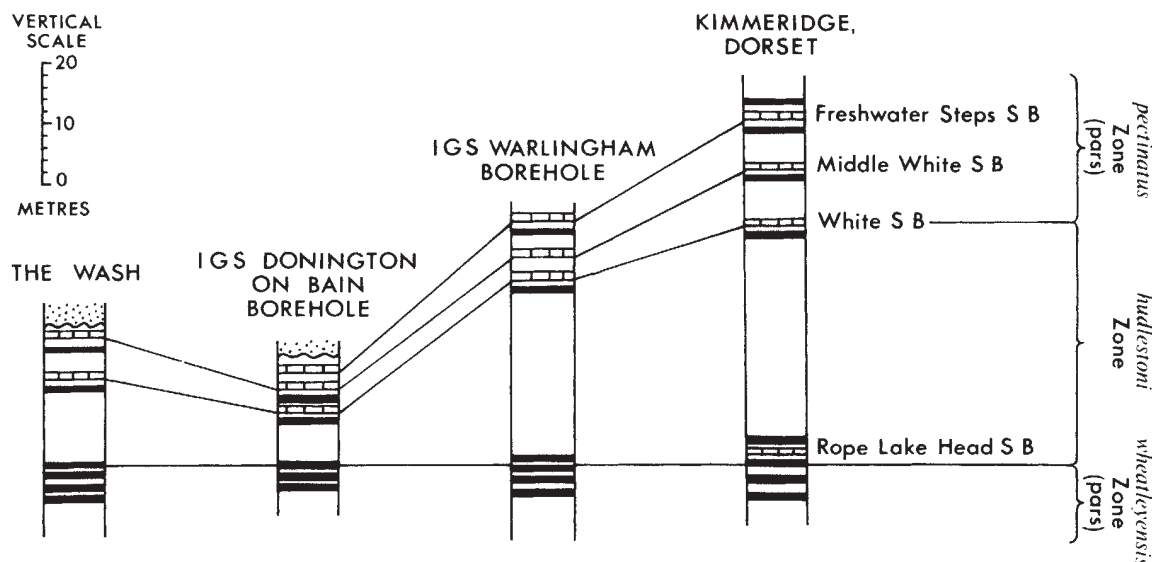


Fig. 1 Correlation of coccolith-rich bands referred to in the text.

highest organic content recorded now in any marine environment is 23.4% in one of the Norwegian fjords. The oil shale fauna, although limited in variety, is fully marine, and clearly does not represent an anaerobic bottom environment comparable to that of any modern closed or restricted basin.

The apparent paradox of substantial amounts of organic material being preserved in sediments which, on general faunal and sedimentary grounds, seem to have been laid down in a shallow, marine environment was first recognised by Hallam⁹ in the Lias. The solution he suggested was that of anaerobic bottom conditions developed beneath a layer of undisturbed, effectively stagnating, seawater^{9,10}.

There are a number of difficulties in applying this suggestion to the oil shales in the Kimmeridge Clay. First, there is evidence of a plentiful benthonic fauna in the oil shales, both in the form of burrowfill structures and bivalve and gastropod shells. Second, the oil shales are thin seams (generally < 0.1 m thick) which occur over a wide area (> 200,000 km²), apparently independent of distance from land and the overall shape of the sea floor. Thirdly, the oil shales occur in juxtaposition (commonly interburrowed) with a variety of lithologies which must represent a variety of differing bottom conditions.

The stagnant bottom conditions hypothesis could explain very local occurrences of oil shale within a single basin of

deposition containing variable bottom conditions, or could explain widespread occurrences within a basin containing uniform conditions. It does not, however, explain the widespread occurrences in the Kimmeridge Clay, which embrace several depositional basins containing variable conditions. Nor does it explain the characteristic association of these oil shales with the occurrences, in flood proportions, of the pelagic flora and fauna of coccoliths, Foraminiferida, ammonites and the crinoid *Saccocoma*.

Dinoflagellate blooms have been recorded from a number of present-day localities, mostly on continental shelves, where their relationship to modern AOM-rich sediments has been studied. The abundance and types of palynomorph present in any given area are governed by complex and interacting factors, such as water turbulence, distance from shore, availability and proximity of river effluence and upwelling¹¹. Such blooms are generally dominated by one or two species and give rise to AOM-rich sediments. The ability of certain types of dinoflagellate bloom to poison the benthonic fauna of an area is well known¹², and it seems likely that if such poisoning took place on a sufficiently large scale, anaerobic bottom conditions, conducive to the formation of pyrite and phosphate, would be temporarily created.

Thus, by analogy with the origin of the coccolith-rich beds,

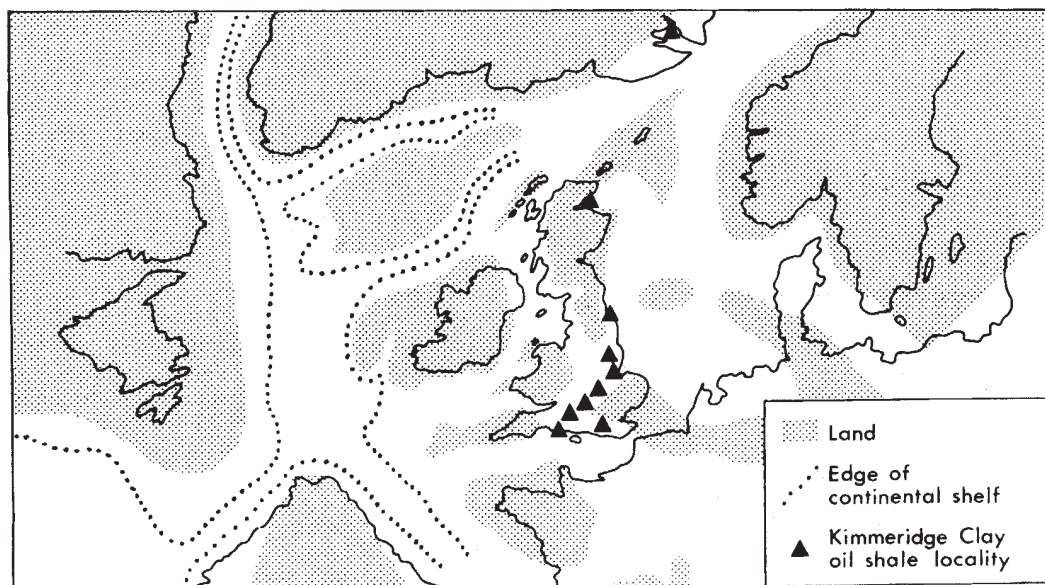


Fig. 2 Palaeogeography in middle Kimmeridge Clay times: continent fit after Laughton¹⁴.

with which they are so intimately associated, the Kimmeridge Clay oil shales appear to have resulted from algal (probably dinoflagellate) blooms. The combined faunal and sedimentary evidence suggests that the organic content of the shales is largely derived from blooms, and that the blooms themselves, by deoxygenating and poisoning the water, provided the temporary anaerobic bottom conditions required to ensure the preservation of their organic content. It is perhaps significant that one of the bivalves most affected by the 1968 toxic bloom off the east coast of Britain was *Lucinoma borealis* Linne¹², and that the bivalve which forms the most extensive plasters in the Kimmeridge Clay oil shales is '*Lucina*' *miniscula* Blake.

Thick oil shale seams occur only in the upper part of the Lower Kimmeridge Clay and the lower half of the Upper Kimmeridge Clay. At these levels the fauna is less diverse than that at lower levels in the Kimmeridge Clay, and seems to represent a less open marine environment. The highest Kimmeridge Clay, above the Rotunda Nodule Bed, contains little bituminous matter and was probably deposited in an even more restricted basin which formed as a result of the Middle Volgian earth movements¹³.

The Kimmeridge Clay oil shales appear therefore to have been deposited in an environment intermediate between open ocean and restricted marine basin at a time when the delicate balance between palaeogeographical influences and nutrient supply was particularly favourable to the formation of algal blooms. The palaeogeography in Kimmeridge Clay times was probably not unlike that of the present-day North Sea, with a series of subsiding basins linked by relatively narrow straits between land areas of low relief (Fig. 2). In the Mesozoic rocks of the British land area, similar palaeogeographical conditions seem to have existed only in the Lias and the Oxford Clay, and the bituminous bands in those formations may have a comparable origin to those of the Kimmeridge Clay.

The likelihood of the Kimmeridge Clay being a major oil source rock off the western coast of Britain would seem to be largely dependent on the nature of the Kimmeridgian palaeogeography. If this area had access to a fully oceanic environment of a proto-Atlantic sea (Fig. 2), then the prospect of thick oil shale seams and a major oil source occurring at this stratigraphical level is unlikely to be high.

I thank Dr A. W. Medd who determined the coccolith percentages.

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Dichotic verbal transformations and evidence of separate processors for identical stimuli

AFTER listening for several seconds to a clear recording of any word or phrase repeated over and over, a listener experiences illusory changes called “verbal transformations” (VTs), and hears a series of abrupt transitions, sometimes to new forms, sometimes back to the actual stimulus or to

illusory forms reported earlier¹. Considerable phonetic distortion may take place, so that with the repeated stimulus ‘tress’ listeners can believe they are hearing clearly words as far removed as ‘purse’ or ‘florist’. The temporary receptive aphasia and perceptual reorganisation produced by VTs have provided access to mechanisms for speech processing not otherwise available for study². Previous investigations have been restricted to one repeated statement at a time; we have extended this research with VTs to investigate dichotic presentation of separate stimuli to each ear.

Our initial observations of dichotic VTs involved presentation of a different repeated word to each ear. We found that subjects had no trouble monitoring both ears, and that VTs seemed to occur in an uncorrelated fashion on both sides. A more interesting condition was suggested by this observation. What if the dichotic stimulus consisted of the same word delivered asynchronously to each ear? By separating the presentation of the single repeated word to each ear by exactly half the duration of the word (a few hundred milliseconds), fusion into a single auditory image would be prevented, and identical stimuli with temporal cross-ear symmetry would be received by each ear. Would VTs occur independently for each side? The answer would have some theoretical interest.

If identical reorganisation to another word occurred at the same time in each ear, it would indicate that a single processor responsible for verbal identification was being used for the input to the two ears. Convergence of dichotic verbal input on the same speech processor has been suggested by some investigators working with the “right ear advantage” effect for speech perception. In studies demonstrating this ear advantage, different verbal messages delivered simultaneously to each ear were shown to be handled asymmetrically, with input to the right ear identified somewhat more accurately^{3,4}. In explanation, it has been suggested that there is competition for access to a single speech processor (located in the left cerebral hemisphere for most right-handed subjects), and that the pathway from the contralateral right ear is favoured over the pathway from the ipsilateral ear^{5,6}.

Alternatively, if VTs with the temporally dichotic but acoustically identical stimuli occurred independently (so that specific repetition-induced phonetic distortions were unilateral), it would suggest that functionally separate verbal decoders or processors were handling the identical input received by each ear. This outcome would raise the possibility that the results of the earlier studies on the right ear speech advantage do not reflect favoured competition for engaging a single processor, but might rather follow from different characteristics of functionally distinct verbal processors, each handling one of the simultaneous messages.

The experimental stimuli were prepared from a single statement of the word ‘tress’ repeated each 492 ms by a loop of recorded tape. A dual output digital delay line was used to split the repeated signal into two identical statements separated by half the word duration (246 ms), and a two-track recording was made with this fixed asynchrony. When played back through matched stereophonic headphones, the identical statements on the two sides were temporally symmetrical, including the contralateral accompaniment for each part of a lateral statement.

Separate groups of college students were used for each experimental condition shown in Table 1, and all (diotic control and three dichotic groups) heard the word ‘tress’ repeated for 5 min. The 20 subjects in the diotic group listened to the repeated word from one of the recorded channels delivered synchronously to each ear. These diotic subjects were unselected for handedness, but because of the correlation between handedness and the side of the dominant speech hemisphere⁷, the 100 subjects used for dichotic listening were all right-handed with no left-handed

Table 1 Verbal transformations with temporally dichotic presentation of a single stimulus

	<i>n</i>	Transitions mean (s.e.)	Forms mean (s.e.)	Proportion of simple changes*
'Tress' (diotic)	20	39.5 (4.90)	6.00 (0.62)	0.418 ± 0.035
'Tress' (dichotic, report one)†				
Right only	40	26.1 (3.38)	5.58 (0.49)	0.289 ± 0.028
Left only	40	23.0 (2.73)	5.50 (0.57)	0.371 ± 0.032
'Tress' (dichotic, report both)†				
Right response	20	14.7 (4.31)	4.15 (0.44)	0.186 ± 0.046
Left response	20	7.1 (1.25)	3.30 (0.26)	0.369 ± 0.086
Total (right + left)	20	21.8 (5.06)	4.85 (0.36)	0.242 ± 0.043

*The range given for proportion of simple changes corresponds to the 95% confidence intervals.

†Delivery to each ear separated by half the duration of the repeated word.

siblings or parents. The instructions were similar to those used in earlier studies⁷. Subjects called out what the voice said, and any changes heard; the experimenter wrote down all responses, later checking the transcription against a tape recording. Forty dichotic subjects were instructed to report only words heard on the right side ('report right'); another 40 were instructed to report only words on the left ('report left'). The final (and most interesting) group consisted of 20 subjects who were instructed to monitor both ears ('report both') and to report all changes, using a hand-operated indicator to show the side on which each reported change occurred. This group did not find it difficult to monitor both ears.

The major finding was that VTs occurred independently on each side. Each of the 20 subjects listening dichotically and monitoring the identical stimuli on both sides reported hearing phonetically different words on the two sides at the same time for some period during the test. As an example, a listener reported hearing the stimulus change from 'commence' back to 'tress' in one ear while the other continued to hear 'tress' repeated over and over. Such independent changes demonstrate that separate speech processors operating up to the level of syllables or words were handling the identical input to each side.

By examining the characteristics of verbal reorganisations under the various experimental conditions, we can compare properties of these processors. In keeping with earlier studies, the results summarised in Table 1 are scored in terms of transitions (perceptual changes) and forms (different perceptual organisations). Individual scores for these two measures are not correlated: as long as an individual reports at least two forms he can achieve a high transition score by rapid changes from one form to the other. After logarithmic transformation⁷, analysis of variance showed that transition scores were significantly higher for diotic listening than for report left, and also significantly greater than left and right combined for report both ($P < 0.05$). There was no appreciable difference between report right and report left only, and for report both, the difference between the right response and the left response, although sizeable, was not significant. The decrease in numbers of illusory changes observed for dichotic relative to diotic listening may involve attentional factors related to the greater complexity and difficulty of the dichotic task. An earlier study¹ indicated that making the task more difficult by the addition of masking noise to a diotic signal also decreased the rate of VTs.

An analysis of variance for numbers of forms showed no significant differences between any of the groups. But if we consider the nature of the changes made by individuals when going from one form to the next, an interesting difference is found. When a listener hears the stimulus word 'tress' change to 'dress' or the illusory word 'frazzle' change to 'trazzle', we have examples of a simple change with one phoneme transformed in each case. Complex changes involve two or more phonemes, and may be as

extreme as the transition reported from 'stress' to 'conniving' in which all phonemes change. The proportion of simple changes for group totals in each experimental condition is shown in Table 1, together with the 95% confidence limits for each proportion. The proportion of simple changes for diotic stimulation was significantly greater than right and left combined for the dichotic report both condition. Within the dichotic report both group, the proportion of simple changes was significantly greater for left than for right reports, as if the left side were more conservative.

Using the asynchronous identical stimuli, it was necessary to introduce each to separate ears to obtain independent VTs. If the outputs of the two stimulus channels were mixed and then introduced diotically to both ears, the mixed signal was not intelligible. With continued listening, the mixed input became organised into a clearly heard single word which then underwent illusory changes characteristic of repeated single words.

Little is known concerning the nature of neural decoding of speech signals; our experiments with dichotic verbal transformations do not inform us of the spatial relation of the separate processors, but merely of their presence and characteristics. It seems that, since more than one processor can be used for identical auditory inputs, we need not consider that the right ear advantage, reported by many investigators for dichotic messages, reflects competition for a single processor as is commonly believed. It seems rather that, if one processor is occupied with a particular task, others may be assembled for similar analyses. The number of potential verbal processors may not be limited to two: in a preliminary experiment with James Bashford we delivered three asynchronous versions of one repeated word through headphones. Each version was preceded and followed by other versions separated by one-third the word duration, with one sequence of the repeated word heard on the left, one on the right and one in the medial plane (the centre signal was delivered diotically; the lateral signals corresponded to separate monaural delays). Monitoring all positions was more difficult with three than with two statements, but all five experienced listeners tested heard the three versions each change independently.

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Correspondence between sharp tuning and two-tone inhibition in primary auditory neurones

THE sharp frequency selectivity of single primary auditory neurones in mammals contrasts with the poor mechanical tuning of the basilar membrane^{1,2}. This discrepancy has generally been explained by postulating some filter mechanism interposed between the basilar membrane and the excitation of auditory afferents²⁻⁴. A further property of these neurones which probably cannot be explained by basilar membrane behaviour alone is two-tone inhibition whereby the simultaneous presentation of a second tone reduces the response of a neurone to a first tone at the best, or characteristic frequency⁵⁻⁸. It has been suggested that this inhibition may also be a property of the additional cochlear mechanisms which follow the basilar membrane⁹. I now present evidence that the loss of sharp frequency selectivity of primary neurones is intimately linked to changes in two-tone inhibition and that the two phenomena might be related to a common mechanism which is vulnerable to perilymph removal.

In 15 guinea pigs anaesthetised with Nembutal, data were collected from 57 single primary neurones in the spiral ganglion of the cochlea¹⁰. The measure of frequency selectivity estimated from the conventional tuning curves was the $Q_{10\text{ dB}}$ value (the characteristic frequency divided by the bandwidth of the tuning curve 10 dB above threshold at the characteristic frequency)⁷. Two-tone inhibition was studied in the same neurones by computing time histograms of the number of action potentials in response to tone bursts. The first tone at the characteristic frequency was presented for 50 ms while the second, inhibiting tone, started 10 ms after the first and lasted for 30 ms (see Fig. 1). Both tones had rise-fall times of 5 ms. The inhibitory regions on either side of the characteristic frequency were mapped out as completely as possible in the time available for each unit. Inhibition was designated "strong", "weak" or "absent" when the second tone, when maximally effective, could reduce the firing rate to the first tone by either more than 50%, by less than 50%, or not detectably, respectively. The first tone was fixed at an intensity of 10-15 dB above the characteristic frequency threshold.

For 33 neurones, the inhibitory regions were thoroughly studied and the strength of inhibition determined by the above criteria and compared with variations in the sharpness of the single-tone tuning curves found from one animal to another^{2,10}. These data, shown in Fig. 1, reveal a close association between the sharpness of neural tuning and the strength of two-tone inhibition.

Furthermore, the removal of perilymph from the scala tympani caused a reversible loss of sensitivity of about 20 dB and a broadening of the tuning curve in cells held throughout the procedure¹¹. In all the cells observed, this effect was accompanied by the disappearance of two-tone inhibition (Fig. 2). Complete data were obtained from four cells in three animals with partial confirmation from other cells. Allowing the perilymph to refill the scala tympani caused a rapid return of sharp tuning and two-tone inhibition. Intensities of characteristic frequency tone less than 10 dB above threshold were tried in the drained cochlea, to see if inhibition was perhaps still present but weak. No inhibition could be demonstrated. A complete range of intensities of the second tone were also used, also

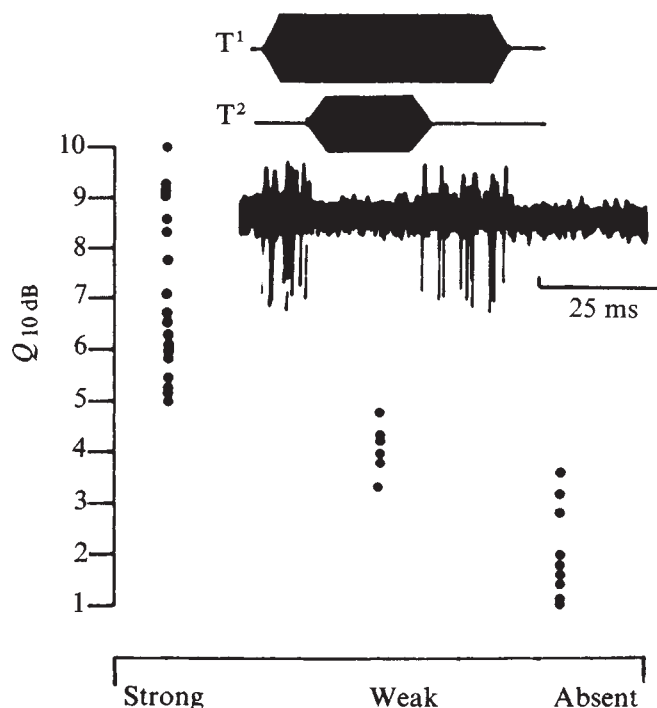


Fig. 1 Tracing shows an example of strong two-tone inhibition in a single spiral ganglion. The approximate positions of the two-tone bursts are shown above the tracing. Three sweeps of the oscilloscope are superimposed. The graph shows the relationship between the sharpness of tuning curves (the $Q_{10\text{ dB}}$) and the strength of two-tone inhibition (see text) for 33 cells in 15 animals.

without demonstrating any appreciable inhibition. Thus two-tone inhibition was totally absent on both the high and low-frequency sides of the abnormally drained tuning curves.

One explanation that should be considered for these results is that the inhibition is lost because the lower slopes of the broadened tuning curves cause an encroachment of the excitatory areas into the inhibitory regions. This is certainly a possibility for the portion of the low frequency slope where the slope changes dramatically during drainage.

For the high frequency slopes, part of the inhibitory regions shown in the tuning curve of Fig. 2 would still fall outside the abnormally broad tuning curve, even if they too were elevated by 20 dB by perilymph drainage. All combinations of characteristic frequency and second tone failed to elicit inhibition. Figure 2b illustrates the only effect that can be produced by the second tone in the drained cochlea. This is an excitatory effect superimposed on the response to the characteristic frequency tone as the intensity of the second tone crosses the boundary of the abnormally broad tuning curve.

Though a more detailed mapping of inhibitory regions than was possible in the present study may reveal some encroachment of the broad tuning curve on to inhibitory regions on the high frequency slope, I believe the present results are sufficient evidence for a genuine loss of two-tone inhibition associated with loss of sharp frequency selectivity.

Thus, loss of sensitivity, loss of inhibition and loss of sharp frequency selectivity seem to go hand in hand. These parallel variations might be explained by alterations in the functioning of a common mechanism. The dramatic and reversible effects of perilymph removal on both frequency selectivity and two-tone inhibition throw some light on the cochlear mechanisms which follow the broad tuning of the basilar membrane. It has been known for some time that the frequency selectivity of primary auditory neurones cannot be related to neurochemical synaptic interactions between

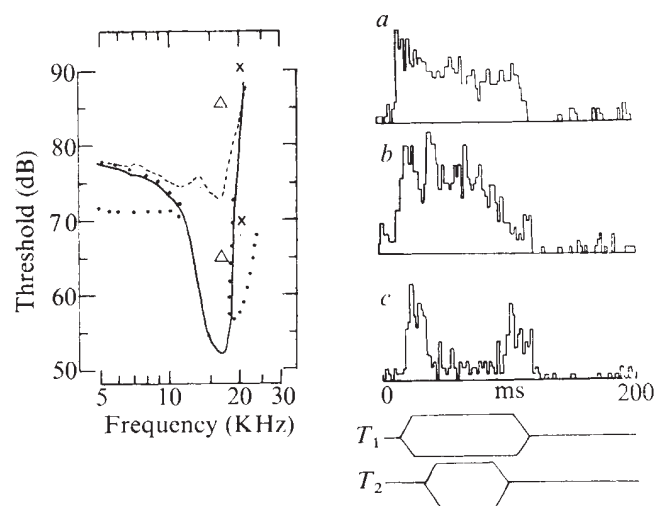


Fig. 2 Graph on left shows the effect of perilymph removal on the tuning curve of a single ganglion cell. Solid line is the tuning curve before drainage, broken line is the curve with perilymph removed. The dotted lines demarcate the inhibitory regions for the cell in the normal case. *a*, *b* and *c* are histograms of the response of the same cell to 20 presentations of characteristic frequency (T_1) and inhibitory tone (T_2). *a*, The initial response to T_1 alone at 17 kHz, 67 dB. *b*, Response with perilymph removed to T_1 now 85 dB and T_2 at 20 kHz, 90 dB. The excitatory effect seen during T_2 presentation is the only form of interaction seen. *c*, Response to the same two frequencies with perilymph replaced. T_1 now 65 dB, T_2 70 dB. Strong inhibition is seen. Sound pressure is measured at the tympanic membrane (dB referred to 0.0002 dyne cm^{-2}).

afferents¹². One has therefore to consider physical mechanisms which could restrict the extent of excitatory input to these neurones arrayed along the cochlear turns. It was originally suggested that the action of perilymph removal might be to alter the mechanical response of the basilar membrane to acoustic stimulation¹¹. Several authors have argued against this^{1,13} and support the alternative explanation; that the underlying mechanism is an increase in the resistance of the scala tympani pathway. Such a resistance change would be expected to interfere with the spread of receptor currents between neighbouring regions of the basilar membrane. Whatever the real explanation turns out to be, the present evidence suggests that both sharp tuning and two-tone inhibition arise from the functioning of such a common mechanism.

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The Red Sea coral *Stylophora pistillata* is an *r* strategist

MACARTHUR and Wilson¹ coined the terms *r* selection and *K* selection to describe two general kinds of selection they believed could be functioning in nature (*K* refers to carrying capacity and *r* to maximal intrinsic rate of natural increase, r_m). As originally defined, the *r* and *K* selection concepts postulated that alternative genotypes within a species possessed somewhat divergent life history characteristics: genotypes with a high r_m were suggested to have a relatively low *K* and vice versa¹. Thus, *r* selection occurs in a fluctuating environment when there is no crowding and it is more important to increase the size of the population. *K* selection occurs in stable environments when population size is always near the maximum and increasing efficiency in resource use is required for the production of a few but extremely fit offspring. Pianka² extended these concepts to include comparisons of individuals of different species. He postulated that no organism is completely *r* selected or completely *K* selected, but all must reach some compromise between the two extremes. Thus, a given species could be visualised along an *r*–*K* continuum and identified by a set of *r* and *K* characteristics².

Stylophora pistillata (Esper), an important scleractinian coral in the Gulf of Eilat³ has most of the *r* characteristics summarised by Pianka²—success in colonising unpredictable reef habitats, rapid development, great population turnover, early reproduction, high r_m , small body size, short life span, density independent mortality (often catastrophic), wide dispersal gradient and poor competitive ability. Most of the other scleractinian corals of Eilat have the opposite characteristics (*K* strategists), or are ranked below *S. pistillata* in their proximity to the *r* end of the *r*–*K* continuum.

The coral reefs of Eilat constitute a physically controlled coral community near the reef flat (the *r* end of the *r*–*K* continuum), existing side by side with the subtidal biologically accommodated community, increasing in diversity (and environmental predictability) to a depth of 50 m (ref. 3). The relative unpredictability of the reef flat at Eilat (in the sense of Slobodkin and Sanders⁴ and Colwell⁵), compared with the deep reef, may be best demonstrated by the catastrophic and extremely low tides which occur periodically but unpredictably along the northern Gulf of Eilat. Thus, in September 1970, an unexpected and extremely low tide caused 80–90% mortality of the hermatypic coral communities on the reef flats of Eilat⁶. In spite of its small colony size³, *S. pistillata* is among the most important

Fig. 1 *S. pistillata* colonies growing on the shell of the gastropod *Trochus dentatus*.



Table 1 Effect of initial size of colony on growth rate

Size interval of radius ($\bar{r}$)*	Number of colonies measured	$\bar{d}_1(\text{cm})^\dagger$	$\bar{d}_2(\text{cm})^\ddagger$	$\frac{\Delta \bar{d}}{\text{yr}}(\text{cm})^\S$	$\frac{\Delta \bar{d}}{\text{yr}}\%$
0.01–0.50	20	0.840 (0.135)	5.104 (0.792)	1.909 (0.254)	227.26
0.51–1.00	52	1.543 (0.267)	7.170 (0.924)	2.520 (0.253)	163.32
1.01–1.50	32	2.536 (0.304)	7.800 (1.168)	2.357 (0.345)	92.94
1.51–2.00	30	3.546 (0.296)	8.958 (0.426)	2.423 (0.271)	68.33
2.01–3.00	32	4.978 (0.530)	10.682 (1.072)	2.554 (0.347)	51.30
3.01–4.00	9	6.892 (0.636)	12.510 (0.650)	2.516 (0.337)	36.50
4.01–5.00	9	8.714 (0.330)	14.055 (1.406)	2.392 (0.276)	27.45

s.d. given in parentheses.

*Geometric mean radius $\bar{r} = (l \times w \times h)^{1/3}/2$.

†Geometric mean diameter at the beginning of measurements.

‡Geometric mean diameter at the end of measurements.

§Mean increase in diameter per year = $365 (\bar{d}_2 - \bar{d}_1)/815$.

||Mean % increase in diameter per year = $\left(\frac{\Delta \bar{d}}{\text{yr}} / \bar{d}_1 \right) \times 100$.

frame builders of the reef flat because of its abundance⁷. Before the catastrophic low tide of 1970, it composed ~25% of the total living coverage of the reef flat of the nature reserve at Eilat. During the low tide, 98% of *S. pistillata* colonies on the reef flat were killed, while other abundant species were somewhat less affected⁶.

In such uncertain conditions, the optimal strategy is to put all possible matter and energy into reproduction, and to produce as many progeny as possible⁸. Histological examination of gonads, in collections made every 2 weeks for a year, revealed that *S. pistillata* breeds during the 8 months from December to July. Colonies 4.0–5.0 cm in diameter already contained ripe eggs⁹. Three years of periodical observations of larval settlement, development and growth indicated that colonies of this size are between 2 and 3 yr old (unpublished). The only other coral species for which estimates have been made of the time required to reach sexual maturity are *Favia doreyensis* (8 yr) and *Fungia actiniformis* (10 yr) in the Great Barrier Reef¹⁰. In general, reduction of the age for first reproduction and an increase in progeny number tend to increase r (ref. 11). Envelopment of *S. pistillata* colonies with plankton nets facilitated the capture of the released planulae, and showed

that a moderate-sized colony may produce 100–400 planulae within 2 h after sunset⁹.

Perhaps the most important feature of *S. pistillata* as an r strategist is that it is usually the pioneer coral species colonising 'new environments'¹ or unexploited habitats. Such species have variously been called 'fugitive'¹², 'opportunistic'¹³, 'colonising'¹⁴, 'weedy'¹⁵, or 'r selected'¹. The seawater system of the Marine Biological Laboratory at Eilat provided a good opportunity to examine the fast-colonising capabilities of this species. Ninety-five colonies of *S. pistillata* were counted in 1972 on the seawater pipe (installed in 1967, 20 m long, 30 cm in diameter), and about 100 colonies on the concrete blocks used to hold down the pipe. Other hermatypic species counted on the seawater system were *Millepora dichotoma* (ten colonies), *Favia fava* (five colonies), *Cyphastrea microphthalmia* (two colonies) and *Acropora variabilis* (one colony). A new seawater pipe, installed parallel to the old pipe was colonised by 45 colonies of *S. pistillata* 8 months later. In April 1975, 92 colonies of this species were recorded, and in August 1975, 173 colonies of this species were counted on the same pipe. So far, no other scleractinian corals have settled on the new pipe. Another demonstration of the opportunistic colonising character of *S. pistillata* is the fact that it may be found growing in shallow lagoons attached to the roots of the mangrove tree *Avicennia maritima*, where water temperature during low tide reaches 36 °C and salinity 48‰. It may also be found attached to the shells of mobile animals, such as the gastropod *Trochus dentatus* (Fig. 1) or to any other newly introduced foreign matter on the reef.

The growth rate of *S. pistillata* is among the fastest of the scleractinian corals in the Gulf of Eilat (Y.L., unpublished and L. Fishelson, personal communication). The growth rate of 184 colonies, of various sizes, of *S. pistillata* was studied during 815 d (May 17, 1973–August 10, 1975). Each colony was numbered by a plastic tag and length, width and height were measured approximately every 3 months. Length is chosen as the distance across a coral between the tips of the branches which are furthest apart. Width is chosen as the distance perpendicular to the length axis. Height is chosen as a measure perpendicular to the length and width axes. When colonies died during the study, I tagged and measured new and healthy colonies of similar dimensions growing nearby. The shape of *S. pistillata* approximates a sphere. The colonies were, therefore, divided into size groups according to the geometric mean of their radius ($\bar{r}$). Although the range of the mean increase in diameter per year ($\Delta \bar{d} \text{ yr}^{-1}$) was very narrow (1.9–2.5 cm), among the size groups studied (Table 1), the

Fig. 2 Healthy colonies of *S. pistillata* (branched colony) and *F. fava* (massive colony) were brought into physical contact on October 7, 1974 (left). On January 1, 1975, the terminal tissues of the branches of *S. pistillata* which touched the *Favia* colony were seen to be killed (right). *S. pistillata* branches within a distance of 2.0–2.5 cm from the *Favia* colony were also killed, which might indicate the furthest distance the mesenterial filaments of *F. fava* can reach. Note that the differences in magnification of the two photographs are due to the use of different lenses of the Nikonos II underwater camera. The left photograph was taken with a 35-mm lens, while the right photograph was taken with a 28-mm lens.



smallest colonies increased in absolute size significantly less compared with the larger ones (t tests, $P < 0.05$). The rate of growth per year of the largest size category, however, was approximately eight times slower than that of the smallest category, which might indicate that like all other organisms they grow proportionately more slowly as they age. The largest colonies of *S. pistillata* do not reach a geometric mean diameter greater than 30–35 cm (ref. 3). It is possible that when a colony reaches the size of sexual maturity (diameter of 4.0–5.0 cm) much of its energy is channelled into the production of sexual cells rather than growth, which considerably lowers the growth rate. Regeneration in this species is also very fast: some accidentally broken branches grew 5.0–7.0 cm yr⁻¹. During this study, 80–85% of the original population of *S. pistillata* died. The causes of mortality were mainly abiotic (winter storms or heavy sedimentation). Nevertheless, many new colonies settled in the same study area. Thus, *S. pistillata* has a short life span and high population turnover in comparison to the other coral species in the same area.

Although the vast majority of the hermatypic corals at Eilat are limited to the deep reef (20–50 m depth) and are rare, *S. pistillata* is very abundant and shows a wide range of dispersal, from the lagoon to the deepest point of the reef⁷. When space becomes limiting, however, *S. pistillata* is the first to be competitively excluded by the deep reef coral specialists.

The area exposed to light is one of the most important limiting factors on a coral reef. Corals compete with each other for available space in various ways¹⁰ one of which takes the form of aggressive behaviour¹⁶. Observations and experiments (Fig. 2) with *S. pistillata* rank this species among the lowest in aggressive hierarchy. That is, on physical contact with most of the other hermatypic corals it is competitively excluded. Abundant species which occur together with *S. pistillata* on the reef flat proved to be more aggressive. Thus, species such as *F. favius*, *Platygyra lamellina*, *Echinopora gemmacea*, *Goniastrea retiformis*, *Pocillopora danae*, *M. dichotoma*, and many others can be seen to extrude their mesenterial filaments and digest away the living tissue of the neighbouring *S. pistillata*. No damaging effects have been observed, however, when several colonies of *S. pistillata* were brought into physical contact with each other.

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Cell differentiation by 3',5'-cyclic AMP in a lower plant

Cyclic AMP evokes or mediates a multitude of responses in animals and microorganisms^{1,2}. Whereas the occurrence and regulatory role of cyclic AMP in these organisms is well established, little is known about its role in plants. Cyclic AMP has been reported to mimic certain effects of gibberellins, indoleacetic acid (IAA) and phytochrome in higher plants but in no case has its endogenous presence been unequivocally demonstrated^{3,4}. We describe here a new response of cyclic AMP at the cellular level in the moss protonema; the results show that it is involved in the differentiation of chloronema cells. The intracellular level of cyclic AMP in these cells is four- to sevenfold higher as compared with that in caulonema cells. In a leaky chloronema-repressed mutant isolated by us, cyclic AMP is shown to enhance the differentiation of chloronema filaments.

The protonema constitutes the juvenile phase in the life cycle of a moss and has been widely used for studies on cell differentiation⁵. In the moss *Funaria hygrometrica*, two distinct cell types are formed during protonema development^{6,7}. A spore germinates producing a multicellular filament. Each chloronema cell contains a large number of chloroplasts and has septa at right angles to the longer axis. Later, caulonema cells are formed; these have fewer chloroplasts and show oblique septa. The buds (initials of moss plants) differentiate only on caulonema cells and their formation is enhanced by cytokinins⁷. The protonema can also be regenerated from any part of a gametophore or a sporophyte, but caulonema cells always differentiate only after the chloronema cells have been formed^{8,9}.

The experiments were carried out with the wild-type cell line J-2 of the moss *Funaria hygrometrica* (L.) Sibth grown in axenic cell suspension cultures¹⁰. In the absence of

Table 1 Effect of cyclic AMP and IAA on the differentiation of chloronema and caulonema filaments

IAA (μ M)	% Chloronema filaments Cyclic AMP (μ M)					
	0	5	10	50	100	500
0	94	97	97	96	96	98
0.5	62	73	80	86	97	—
1.0	33	52	73	68	91	93
2.0	33	45	52	61	—	—
5.0	21	30	39	49	—	—

Cells were cultured in minimal medium (MM) at $26 \pm 2^\circ \text{C}$ in continuous light at an initial cell density of 20 mg l^{-1} as described previously¹⁰. Cyclic AMP sterilised by filtration through membrane filters was added to the autoclaved MM $\pm$ IAA. After 6 d, at least 500 filaments were scored to determine the proportion of chloronema filaments.

exogenous auxins, protonema grown in liquid culture consist predominantly of chloronema filaments. Auxins enhance the differentiation of caulonema filaments¹¹. In cultures grown in the presence of IAA, adenine derivatives other than cytokinins were observed to enhance the differentiation of chloronema filaments, thus antagonising the effect of IAA.

Formation of chloronema filaments was profusely enhanced by cyclic AMP and at a concentration of $100 \mu\text{M}$, their percentage was restored equal to that in control (Table 1). This increase was due to initiation of a large number of new chloronema branches and not due to a dedifferentiation of existing caulonema filaments. The latter continued to produce caulonema cells and this result strongly suggests that caulonema differentiation is independent of cyclic AMP.

Table 2 Effect of various nucleotides and inhibitors of phosphodiesterase on the formation of chloronema and caulonema filaments in wild-type and mutant *pg-1* protonema

Compound	% Chloronema filaments Wild type	<i>pg-1</i>
Nucleotides		
None	35	27
3',5'-cyclic AMP	91	66
Monobutyl cyclic AMP	91	37
Dibutyl cyclic AMP	78	45
Cyclic GMP	78	41
Cyclic IMP	59	35
Cyclic CMP	40	26
Adenosine	71	24
5'-AMP	59	24
5'-GMP	81	26
Phosphodiesterase inhibitors		
Theophylline	85	40
Aminophylline	95	42
ICI 58,301*	88	42

*3-Acetamido-6-methyl-8-*n*-propyl-s-triazolo[4,3a]pyrazine.

Mutant *pg-1* was isolated as follows. Spores (1.52×10^5 per 3 ml) suspended in MM+0.001% Tween-20 were exposed for 45 min to ultraviolet light from a Philips germicidal lamp (1.25% of the spores survived). After exposure, spore suspension was stored in dark (30 h) and then plated on complete medium. The protonema that grew 0.5 cm or more in diameter in 10 d (650) were screened for altered differentiation of various cell types. Three isolates behaved differently and only in one of these (designated *pg-1* here) caulonema filaments were found to be formed in the absence of IAA. The detailed method of isolating mutants will be published separately. Wild-type protonema was grown in MM+1 μ M IAA, whereas *pg-1* was cultured in MM+1% glucose. All nucleotides and inhibitors of phosphodiesterase were sterilised by filtration through membrane filters and then added to the autoclaved medium. A wide range of concentrations were tested; data are given only for 0.1 mM. Other details as in Table 1.

Mono- and dibutyl cyclic AMP, purine nucleotides and inhibitors of cyclic nucleotide phosphodiesterase also enhanced chloronema differentiation (Table 2). Theophylline and aminophylline, although active, inhibited the growth of cells at all the concentrations tested (0.01–1 mM). ICI 58,301 (an inhibitor of phosphodiesterase¹²) was not inhibitory and mimicked the effect of cyclic AMP. The pyrimidine derivatives were completely inactive. The compounds enhancing chloronema differentiation in the wild type may act by elevating endogenous levels of cyclic AMP, and an increase in the activity of adenylate cyclase by most of these has been shown in animal systems^{13,14}.

Further evidence about the specific involvement of cyclic AMP in chloronema differentiation comes from the results obtained with the pale green mutant (*pg-1*), which was isolated by treating the wild-type spores (cell line J-2) with ultraviolet irradiation as described in Table 2. In suspension cultures, this mutant produced predominantly caulonema filaments (>70%) without the addition of an auxin (the

rest being the chloronema filaments). It may be recalled that in the wild type very few (<10%) or no caulonema filaments are formed in the absence of an auxin. In the mutant *pg-1*, cyclic AMP was quite active, whereas dibutyl cyclic AMP and phosphodiesterase inhibitors were marginally active (Table 2). Six days after addition of 0.1 or 1 mM cyclic AMP, a maximum of 66–70% chloronema branches were seen (control showed 27%). These branches arose as laterals on caulonema filaments and appeared similar in all respects to such branches from the wild-type protonema. Adenosine, 5'-AMP, 5'-GMP and other cyclic nucleotides did not alter the relative proportion of caulonema and chloronema filaments.

To elucidate the role of endogenous cyclic AMP, its intracellular levels were determined in the wild-type and the mutant protonema using the protein kinase assay¹⁶ (Table 3). Detailed results concerning the characterisation of cyclic AMP in this moss will be published elsewhere. The endogenous concentration of cyclic AMP was four- to sevenfold higher in chloronema cells compared with a population of cells consisting of about 65–75% caulonema filaments. Cyclic AMP levels in the mutant and in the wild-type caulonema filaments were the same. A several-fold greater level of intracellular cyclic AMP in chloronema cells compared with that in caulonema filaments, and their enhanced differentiation by exogenous cyclic AMP, strongly suggest that cyclic AMP has a role in chloronema differentiation. The enhancement of chloronema filaments by adenosine and 5'-AMP in the wild-type protonema only (and not in the mutant) is a significant difference. Our results are consistent with the *pg-1* mutant being at least partially impaired in the synthesis of cyclic AMP.

Our results show that cyclic AMP enhances the differentiation of chloronema cells, whereas the auxins are already known to inhibit their growth and differentiation^{11,17,18}. The chloronema cells contain IAA (M.M.J., unpublished), and a balance of endogenous cyclic AMP and auxin may in fact underlie the differentiation of chloronema cells. The results of the experiment in which the amount of cyclic AMP and auxin were altered in the medium support this suggestion; also, the percentage of chloronema filaments was greater at high cyclic AMP–IAA ratios.

The widespread occurrence of cyclic AMP among heterotrophic organisms¹ has led many investigators to test for its presence and function in plants^{3,4}. So far, cyclic AMP or enzymes of its metabolism have been found in bacteria², fungi^{19–22} and an alga²³, but not in higher plants. In the context of our results on the moss protonema, the situation in lower plants may indeed be different from that of higher plants. Based on available evidence, cyclic AMP seems to be a naturally-occurring regulator of growth and differentiation only in the lower plants.

Table 3 Endogenous levels of cyclic AMP in wild-type and mutant protonema

Predominant cell type	Medium	% Chloronema	Cyclic AMP (pmol) per g fresh weight	per mg protein
Wild type				
Chloronema	MM	100	56	2.05
	MM+1% glucose	100	44	2.23
Caulonema	MM+1 μ M IAA	35	8	0.53
Mutant <i>pg-1</i>				
Caulonema	MM+1% glucose	25	5	0.53

Chloronema cells were grown in MM+1% glucose and collected in late exponential phase, whereas cultures of caulonema filaments were obtained by growing chloronema cells in MM+1 μ M IAA for 6 d. Mutant grew better in MM+1% glucose and cells were collected after 5 weeks.

For cyclic AMP determination, cells were homogenised in 6% Cl_3CCOOH in a blender at 4 °C. ^3H -cyclic AMP (3,000 c.p.m., specific activity 20.8 Ci mmol⁻¹) was added as internal standard and homogenate was centrifuged at 27,000g for 15 min. Pellet was saved for determination of protein¹⁵ and cyclic AMP was determined in the supernatant. Supernatant was extracted five times with cold water-saturated diethyl ether and the resulting aqueous phase lyophilised after removal of ether. Dry material was dissolved in distilled water and chromatographed on BioRad Aminex MS cation exchange resin (mesh size 200–400). Cyclic AMP-containing fractions were freeze dried and chromatographed on thin-layer silica gel plates in *n*-butanol–methanol–ethylacetate–ammonia (7:3:4:4, v/v). Area corresponding to cyclic AMP was eluted with 50% ethanol and eluate lyophilised. Purified material was dissolved in distilled water and cyclic AMP determined using protein kinase assay as described by Kuo and Greengard¹⁶ except that the source of protein kinase was rabbit skeletal muscle. Cyclic AMP levels in the samples of caulonema cells (wild type and *pg-1*) were three- to fivefold greater than the lowest amount of cyclic AMP detectable (0.5 pmol) in our assay conditions.

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Adenylate cyclase activity and steroidogenesis in phenotypic revertants of an ACTH-insensitive adrenal tumour cell line

A LARGE body of evidence implicates adenylate cyclase and cyclic AMP as mediators of adrenocorticotrophic hormone (ACTH)-stimulated adrenal steroidogenesis (see ref. 1 for review). Recent observations, however, suggest that ACTH acts at separate sites to increase steroidogenesis and cyclic AMP accumulation, and that cyclic AMP may not be an important component of ACTH-stimulated steroidogenesis^{2–5}. An ACTH-responsive adrenocortical tumour cell line⁶ (clone Y1) and a derivative clone insensitive to ACTH⁷ (clone Y6), have been used to examine the role of adenylate cyclase and cyclic AMP in adrenal steroidogenesis^{1,7}. Y6 cells do not respond to ACTH with increased steroidogenesis but do respond to added cyclic AMP⁷. In addition, these cells have an adenylate cyclase system insensitive to ACTH, probably accounting for their variant phenotype¹. Y6 cells, when grown as tumours, regain the ability to respond to ACTH with increased steroidogenesis, and continue to express the ACTH-sensitive phenotype after prolonged periods in culture and extensive dilution⁷. Y6 cells, after animal passage, provide a system of phenotypic revertants to examine the linkage of different biochemical events to ACTH insensitivity^{7,8}. We have now examined adenylate cyclase activity in phenotypic revertants of Y6 cells induced by animal passage to assess the role of this enzyme in steroidogenesis. After animal passage, Y6 cells regained the ability to respond to ACTH both with increased steroidogenesis and with increased adenylate cyclase activity. These data indicate that the actions of ACTH on adenylate cyclase activity and steroidogenesis are closely linked.

Y6 cells were grown in monolayers or as tumours in isogenic mice (LAF₁; Jackson Laboratories) as described previously⁷. For steroid assays, cells were incubated in growth medium containing 2.5 mM CaCl₂, and 0.1% trypsin inhibitor (from lima bean) for 1 h. At the end of the incubation periods steroids

were extracted from the growth medium in methylene chloride and measured against a corticosterone standard by fluorescence⁹. Adenylate cyclase activity was determined by measuring the conversion of labelled ATP to labelled cyclic AMP as described previously¹. Labelled cyclic AMP was separated from other labelled compounds by chromatography on Dowex 50 and treatment with BaSO₄ (ref. 10). Cells were screened for aerobic and anaerobic bacteria to ensure that they were not contaminated. Mycoplasma infections were demonstrated by characteristic growth in broth and in agar¹¹. Mycoplasma were determined by Dr P. Quinn, Hospital for Sick Children, Toronto.

Y6 cells infected with mycoplasma¹ tentatively identified as *M. arginitia* (E. Bransome, personal communication) regained the ability to respond to ACTH with increased steroidogenesis when grown as tumours and plated as primary cultures (Table 1 and ref. 7). Concomitantly, the tumours and primary cultures recovered the ability to respond to ACTH with increased adenylate cyclase activity (Table 2). ACTH-sensitive adenylate cyclase activity was observed in intact cells, homogenates and membrane particles prepared from primary cultures of Y6 tumours (Table 2). Clonal isolates from primary cultures of Y6 cells after animal passage responded to ACTH and to cyclic AMP with increased steroidogenesis (Table 1). Homogenates and membrane particles prepared from these clonal isolates also responded to ACTH with increased adenylate cyclase activity (Table 2). Thus after animal passage, the same cell recovered the ACTH-sensitive phenotype both in steroidogenesis and adenylate cyclase activity. F[−] stimulated adenylate cyclase activity in preparations from primary cultures and to a greater extent in preparations from clonal isolates (Table 2). After animal passage, these cells no longer carried the mycoplasma infection.

Y6 cells cured of mycoplasma infection *in vitro* with kanamycin sulphate remained insensitive to ACTH¹. After animal passage, the cells treated with kanamycin sulphate did not recover ACTH sensitivity with respect to steroidogenesis or adenylate cyclase activity (data not shown). When the cured cells were reinfected with mycoplasma and grown as tumours, they regained the ability to respond to ACTH with increased steroidogenesis (Table 1) and increased adenylate cyclase activity (Table 2). ACTH-responsive Y1 cells retained ACTH sensitivity when infected with mycoplasma and grown for several months (data not shown) or when treated with kanamycin sulphate¹.

As demonstrated here, phenotypic reversion of Y6 cells requires the combination of mycoplasma infection and animal

Table 1 Steroidogenesis in Y6 cells after animal passage

Cell population	Steroids secreted (ng h ^{−1} per mg protein)		
	Unstimulated	+ACTH	+cyclic AMP
Experiment 1			
Primary culture	170 ± 15	710 ± 35	Not determined
Clone 1	80 ± 5	280 ± 30	215 ± 20
Clone 2	110 ± 5	580 ± 60	370 ± 15
Experiment 2			
Primary culture	200 ± 10	750 ± 50	Not determined

Y6 cells were grown as solid tumours in LAF₁ male mice and plated as primary monolayer cultures *in vitro*⁷. Clones were isolated by plating single cells from primary cultures in microwells for tissue culture. Where indicated, ACTH (ACTHar, Parke Davis) was added at 5 mU ml^{−1} and cyclic AMP at 1 mM. In experiment 1 tumours were formed from Y6 cells infected with mycoplasma. Before animal passage, these cells secreted 180 ± 20 ng steroid per h per mg protein without ACTH; and 250 ± 15 ng steroid per h per mg protein with ACTH. In experiment 2 Y6 cells (approximately 10⁶ cells per 75 cm² flask), cured of mycoplasma *in vitro* with kanamycin sulphate¹, were reinfected by adding 0.1 ml of medium from mycoplasma-infected Y6 cells to 15 ml of culture medium. The infected medium was centrifuged at 200g to remove any floating cells before use. Cells were cultured for 2 weeks with mycoplasma titres reaching approximately 10⁷ colony forming units in agar per ml of medium, and then injected into mice.

Table 2 Cyclic AMP accumulation in Y6 cells after animal passage

Cell population	Cyclic AMP accumulation		
	Unstimulated	+ACTH	+F ⁻
Experiment 1			
Solid tumour particles	30±5	110±5	—
Primary cultures			
Intact cells	2,030±430	4,900±160	—
Homogenates	100±10	950±70	2,330±120
Particles	65±5	165±10	3,130±210
Clone 1			
Homogenates	40±5	660±50	3,730±170
Particles	110±10	810±10	10,050±70
Clone 2			
Homogenates	80±10	270±10	4,480±70
Particles	110±5	240±10	7,780±160
Experiment 2			
Solid tumour particles	26±1	127±1	—

Cell populations were obtained for experiments 1 and 2 as described in Table 1. Adenylate cyclase in intact cells was determined by measuring the conversion of endogenous labelled ATP to labelled cyclic AMP as described previously¹ but without theophylline. ACTH was added to intact cells at 500 mU ml⁻¹. The results for cyclic AMP accumulation in intact cells are expressed as c.p.m. per 30 min per mg protein ±s.e.m. Cell homogenates and membrane particles were prepared and assayed for enzyme activity as described previously¹. ACTH (ACTHar, Parke Davis; 625 mU ml⁻¹) and NaF (15 mM) were added where indicated. The results for cyclic AMP accumulation with homogenates and membrane particles are expressed as pmol per 10 min per mg protein ±s.e.m. Adenylate cyclase activity in homogenates of Y6 cells before animal passage was 30±5 pmol per 10 min per mg protein without ACTH; and 40±5 pmol per 10 min per mg protein with ACTH.

passage. Although the mechanism of restoration of ACTH sensitivity is not understood, these results suggest an unusual mechanism for the regulation of phenotypic expression. The possibility that this phenomenon is related to true genetic reversion remains open, since previous studies have demonstrated that mycoplasma can induce permanent phenotypic and karyotypic changes in cell cultures¹².

Nevertheless, the concomitant recovery of ACTH-sensitive phenotypes in steroidogenesis and adenylate cyclase activity after animal passage demonstrate that the two events are closely linked. These observations indicate that the inability of Y6 cells to respond to ACTH with increased adenylate cyclase activity and with increased steroidogenesis is the result of a common defect, and suggest that at least one component of the ACTH-sensitive adenylate cyclase system is essential for the action of ACTH on adrenal steroidogenesis.

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Inhibitory action of noradrenaline and cyclic AMP in explants of rat cerebellum

IZANTOPHORETICALLY applied noradrenaline (NA) produces sustained depression of spontaneous firing of rat cerebellar Purkinje cells^{1–3} and this inhibition may be mediated by cyclic AMP^{4–5}. This contention has been challenged by Godfraind and Pumain⁶ as well as by Lake and Jordan³ who failed to observe a depressant action of cyclic AMP. Because the results may be influenced by electrical current or pH artefacts, the kind of anaesthetics used or factors impeding the release of cyclic AMP from micropipettes⁷ we have used cultured Purkinje cells from rat cerebellum^{8–10} to test the hypothesis that cyclic AMP mediates the depressant action of NA on Purkinje cells. We found that high concentrations of cyclic AMP only slightly decreased the firing rate of Purkinje cells, but that low concentrations of inhibitors of phosphodiesterase strongly enhanced the inhibition induced by cyclic AMP and NA.

Explants of cerebellum were cultured from newborn rats by the roller tube technique^{11,12}. All electrophysiological experiments were conducted during the third week in culture. Single unit spontaneous action potentials were recorded extracellularly from large nerve cells (presumably Purkinje cells) with microelectrodes filled with 2 M NaCl¹². The drugs were added directly to Hanks' balanced salt solution which served also as control solution.

When the monoamine was added to the bathing fluid in low concentrations, NA, like isoproterenol⁹, altered the pattern of spontaneous discharges by selectively increasing the number of long interspike intervals. Higher concentrations (>10⁻⁵ M) slightly decreased the firing rate and the cells stopped firing at a concentration varying between 10⁻⁴

Table 1 Response of cultured Purkinje cells to various drugs applied in the bathing fluid

Substance	n	+	–	–
NA 10 ⁻⁵ M	14	29	14	57
NA 10 ⁻⁴ M	11	18	27	55
cyclic AMP 10 ⁻⁴ M	11	18	18	64
cyclic AMP 10 ⁻³ M	23	17	44	39
AMI 10 ⁻⁴ M	10	—	40	60
AMI 10 ⁻³ M	12	—	83	17
PAP 10 ⁻⁶ M	6	—	—	100
PAP 10 ⁻⁵ M	7	—	100	—
AMI 10 ⁻⁴ M + cyclic AMP 10 ⁻⁴ M	19	—	79	21
AMI 10 ⁻⁴ M + NA 10 ⁻⁵ M	10	—	70	30
AMI 10 ⁻⁴ M + NA 10 ⁻⁴ M	16	—	100	—
PAP 10 ⁻⁶ M + NA 10 ⁻⁴ M	6	—	100	—
cyclic AMP 10 ⁻⁴ M + NA 10 ⁻⁴ M	8	13	37	50
cyclic AMP 10 ⁻³ M + NA 10 ⁻⁴ M	8	—	75	25

Results are expressed as percentage of tested cells. n, Number of cells tested; +, excitation; –, inhibition; –, change in rate ≤ 10%; AMI, aminophylline; PAP, papaverine.

and 10⁻³ M. The action of NA was, compared with other substances such as gamma aminobutyric acid⁸ or glutamate¹⁰, slow in onset and only partially reversible. Only before the cessation of the activity did the amplitude of the spikes decrease.

Cyclic AMP had no effect at 10⁻⁴ M. At 10⁻³ M, the firing rate of 44% of the cells was decreased (Table 1), but no cells stopped firing. With monobutyl or dibutyl cyclic AMP, used on 14 cells (not shown in Table 1), there was no significant difference in response. Even in the absence of exogenous cyclic AMP, inhibitors of phosphodiesterase, believed to increase the intracellular cyclic AMP concentration, consistently depressed the firing of Purkinje cells (Fig. 1a). Papaverine was about 100 times more potent than aminophylline and caffeine in abolishing the

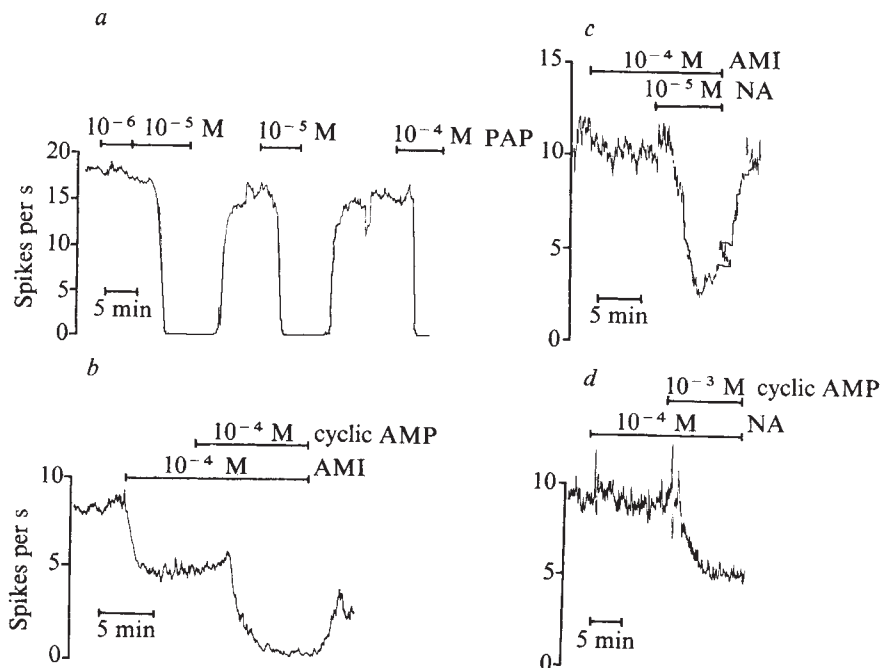


Fig. 1 Examples of the effects of various drugs on the rate of spontaneous discharge of Purkinje cells. *a*, Application of 10^{-5} M papaverine abolished firing. After removal of the drug, the cells resumed firing. The effect of 10^{-4} M papaverine was, however, only very slowly reversible. *b*, The inhibition induced by 10^{-4} M aminophylline was enhanced by a subthreshold concentration of cyclic AMP. *c*, In the presence of aminophylline, 10^{-5} M NA produced sustained depression of firing. In this cell, the rate of firing was not decreased by application of 10^{-4} M aminophylline. *d*, High concentrations of NA and cyclic AMP slowed, but did not stop, firing of Purkinje cells.

spontaneous discharges (10^{-3} M of the latter were about equiactive with 10^{-5} M papaverine).

The efficacy of various substances in potentiating the inhibitory effect is shown in Table 1 and examples of drug responses are illustrated in Fig. 1. The inhibition produced by 10^{-4} M aminophylline was enhanced by a subthreshold concentration of cyclic AMP (Fig. 1*b*). On a cell which was virtually unaffected by 10^{-4} M aminophylline, the addition of 10^{-5} M NA caused sustained depression of firing (Fig. 1*c*). Surprisingly this inhibition was rather quickly reversed after removal of the drugs. Concomitant addition of 10^{-4} M NA and 10^{-3} M cyclic AMP inhibited Purkinje cells only to some degree (Fig. 1*d*), whereas most cells exposed to 10^{-4} M NA and 10^{-4} M aminophylline stopped firing.

Cultured Purkinje cells probably retain receptors for NA, and their activation could have been responsible for the inhibition in the presence of NA. The need for relatively high concentrations of NA to elicit an effect could be due to a relatively small number of intact receptors or, if the second messenger hypothesis of cyclic AMP is accepted, to high activity of phosphodiesterase. The latter might also be responsible for the failure of cyclic AMP to produce a clear, sustained depression of firing.

The fact that NA and inhibitors of phosphodiesterase produced similar electrophysiological results does not directly prove or disprove a link between their actions, but the hypothesis is strengthened because the action of NA was enhanced by inhibitors of phosphodiesterase. Substances that selectively inhibit brain adenylate cyclase might provide a means to determine whether cyclic AMP is the second messenger for NA in cerebellum. Lead was reported to inhibit selectively adenylate cyclase in rat cerebellum¹³. Unfortunately, application of lead (10^{-9} – 10^{-4} M) in our system led to ambiguous results, sometimes preventing, sometimes enhancing NA-induced inhibition.

In conclusion, phosphodiesterase activity seems to be high in cultured cerebellum. This might explain the weak effect of cyclic AMP (and possibly also of NA) on Purkinje cell firing. Inhibitors of phosphodiesterase strongly enhanced the inhibition produced by NA and cyclic AMP. The data reported support the hypothesis of Bloom *et al.*^{4,5,14} that the intracellular level of cyclic AMP influences noradrenergic neurotransmission.

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Allergic sensitisation of human lung fragments prevented by saturation of IgE binding sites

PASSIVELY sensitised chopped human lung fragments release mediators of immediate hypersensitivity when challenged with appropriate antigens¹. Antigen triggers the release of mediators from mast cells or basophils by coupling of neighbouring surface IgE antibody molecules sharing the same antigen specificity². Release of mediators should therefore be unlikely if specific IgE molecules are sparsely distributed on cell surfaces, as in normal non-allergic people, or if surface receptors are densely occupied by IgE molecules with other specificities. Humans harbouring certain parasites develop high IgE levels³, and epidemiological evidence suggests that allergic disorders such as hayfever or pollen asthma are rare in heavily parasitised populations^{4,5}. It is not known whether IgE induced in man by parasite infestation is directed specifically against parasite antigens, or whether it represents nonspecific potentiation of IgE production against many different antigens, as demonstrated in the rat *Nippostrongylus* model⁶. Here we show that human lung fragments first exposed to IgE-rich serum from West African residents become resistant to further passive

Table 1 Effect of IgE-rich serum on subsequent passive sensitisation and challenge

Preincubated with:	Lung fragments Sensitised with:	Challenged with:	No. of experiments	Mean histamine release (ng per g tissue, mean ± s.d.)
West African IgE-rich serum	Allergic serum	Pollen antigen	13	282 ± 92
Cord* serum	Allergic serum	Pollen antigen	13	2,316 ± 435
Cord serum	Allergic serum	Cord serum	13	171 ± 27
Cord serum	Cord serum	Pollen antigen	13	222 ± 33
West African IgE-rich serum	Cord serum	Pollen antigen	3	165 ± 54

*Cord serum used in control experiments contained less than 1 U ml⁻¹ of IgE.

sensitisation with serum containing grass pollen-specific IgE.

Normal tissue obtained from peripheral lung lobes removed at operation was finely chopped, filtered on gauze and washed three times in Krebs solution. Aliquots (200 mg) were incubated for 90 min at 37 °C in pooled serum, diluted 1:100 in Krebs solution, from 8 West African subjects, residents in Manduar, The Gambia. The IgE level of this pool was 6,454 U ml⁻¹, measured by double antibody radioimmunoassay⁷ (1 U = approximately 2.4 ng (ref. 8)). The treated aliquots of lung tissue were washed three times in Krebs solution and resuspended in serum, diluted 1:100, from a patient with hayfever. The total IgE of this second serum was only 60 U ml⁻¹, but high specific IgE antibody activity to Timothy grass pollen was demonstrated by the

a 73% blocking effect, comparing histamine release from blocked lung fragments with that from challenged control fragments exposed only to cord serum before passive sensitisation. Similar results were obtained when other allergic sera from different patients were used for passive sensitisation.

Table 2 shows the results of experiments designed to confirm that the blocking effect was related specifically to IgE antibody. All inhibitory activity was lost after heating the IgE-rich serum at 55 °C for 30 min, enough to denature IgE but not other immunoglobulins. Furthermore, lung fragments exposed to unheated IgE-rich serum, although refractory to further passive sensitisation, released histamine readily on treatment with anti-IgE.

Table 2 Effect of heat treatment, and of challenge with anti-IgE

Preincubated with:	Lung fragments Sensitised with:	Challenged with:	No. of experiments	Mean histamine release (ng per g tissue, mean ± s.d.)
Cord serum	Allergic serum	Pollen antigen	3	2,164 ± 184
Untreated IgE-rich serum	Allergic serum	Pollen antigen	3	238 ± 21
Heat-treated IgE-rich serum†*	Allergic serum	Pollen antigen	3	2,170 ± 240
—	IgE-rich serum	Anti-IgE†	2	2,998 ± 536
—	Allergic serum	Anti-IgE	2	3,435 ± 697
—	Cord serum	Anti-IgE	2	238 ± 32

*Heated at 55 °C for 30 min.

†Anti-IgE was prepared in a sheep immunised against N.D. myeloma IgE; the IgG fraction was separated from sheep serum by 3 ammonium sulphate precipitations, followed by DEAE-cellulose chromatography.

radio-allergo-sorbent technique⁹. After overnight incubation at room temperature and a further 60 min at 37 °C the lung fragments were washed and challenged with freeze-dried extract of Timothy grass pollen in Krebs solution (100 µg ml⁻¹) for 15 min. Histamine released into the supernatant was measured by bioassay, using strips of guinea pig ileum bathed in Krebs solution at 37 °C containing atropine sulphate 60 µg ml⁻¹.

Table 1 shows the combined results of experiments in which previous exposure of lung fragments to IgE-rich serum effectively blocked subsequent passive sensitisation. There was some variation in histamine released by different lung specimens, but in no experiment was there less than

Finally, the effect of altering the experimental sequence was studied (Table 3). When lung was first passively sensitised with serum containing grass-pollen specific antibody, subsequent exposure to West African IgE-rich serum failed to interfere with histamine release after challenge. Nor was significant blocking found when lung fragments were exposed to grass-pollen specific antibody and IgE-rich serum simultaneously, in spite of a total IgE level 100-fold higher in the West African serum than in the allergic serum.

These results support the hypothesis that IgE receptors, numbering up to 100,000 per mast cell¹⁰, can be saturated, thus making the cell unresponsive to further attempts at passive sensitisation¹¹. In this model system blocking was

Table 3 Effect of altering the experimental sequence

Preincubated with:	Lung fragments Sensitised with:	Challenged with:	No. of experiments	Mean histamine release (ng g ⁻¹ tissue)
IgE-rich serum	Allergic serum	Pollen antigen	3	238 ± 21
Allergic serum	IgE-rich serum	Pollen antigen	3	2,348 ± 295
—	Mixed allergic serum and IgE-rich serum*	Pollen antigen	4	1,841 ± 173

*The final dilution of both sera was 1:100.

achieved only if IgE-rich serum was added to lung in a separate step before passive sensitisation with allergic sera, results which are in keeping with the known high affinity binding of IgE to mast cell surface receptors. Mast cell blockade through saturation of IgE receptors is also suggested by the failure of patients with IgE myeloma to accept passive sensitisation¹², and by animal experiments^{13,14}. Now it is necessary to know whether IgE produced actively by parasite infection or by the administration of parasite derived antigen can displace IgE against environmental allergens already existing in an allergic patient.

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Suppression of bacterially-induced hypersensitive reaction and phytoalexin accumulation in bean by phaseotoxin

PHASEOTOXIN is a trivial name given to an exotoxin produced by *Pseudomonas phaseolicola*, which causes halo blight of beans¹. Although the mechanism of chlorosis induction in susceptible hosts is unknown, evidence indicates that chlorosis is casually related to inhibition of ornithine carbamoyltransferase (OCT; EC 2.1.3.3) by phaseotoxin, a potent and specific inhibitor of the enzyme². Phaseotoxin may also be involved in the host specificity of the pathogen. We previously reported that in infected resistant bean plants (but not in infected susceptible ones) phaseotoxin production is suppressed in spite of substantial bacterial multiplication³. Further, in resistant beans treated with phaseotoxin, a larger number of bacteria are found than in non-treated plants⁴. These observations indicate that phaseotoxin may suppress the hypersensitive reaction (HR) in resistant hosts. The object of this study was to investigate whether pretreatment of resistant cultivars of bean with phaseotoxin suppresses the HR response of the host and phytoalexin accumulation on subsequent inoculation with *P. phaseolicola*, and our results seem to support this.

The term HR is applied to an acute, localised response of plant tissues to inoculation with incompatible pathogens. In plants infected (or inoculated) with incompatible fungal pathogens, HR is accompanied by accumulation of phytoalexins, compounds which are toxic to the invading pathogens. There are only two examples of HR induction and phytoalexin accumulation in plants infected with incompatible bacterial pathogens^{5,6}. The compounds which occur in soybeans inoculated with *P. glycinea* have been identified⁵ and we have recently identified some of those which accumulate in beans infected with *P. phaseolicola*^{7,8} and determined their toxicity to the pathogen.

Seedlings (6 d old) of a resistant bean cultivar were

allowed to take up nutrient solution plus phaseotoxin (4,200 U ml⁻¹) or nutrient solution alone (controls), in a growth chamber for 24 h. At the end of the incubation period the leaves of treated plants contained approximately 1,300 U of phaseotoxin per 2 cm² leaf area. The plants were then replanted in vermiculite and the abaxial surfaces of their primary leaves inoculated with a suspension of *P. phaseolicola*. Leaf disks (2 cm²) were removed every 24 h from the toxin-treated and control plants and their bacterial populations determined as previously described³.

In control plants the number of bacteria found per disk were 7.1×10^7 and 1.2×10^8 cells 2 and 4 d after inoculation, whereas in toxin-treated plants the bacterial numbers were 6.7×10^6 and 3.3×10^{11} cells for the same periods (Fig. 1). After 3 d, the control plants responded with HR, exemplified by tissue silvering, collapse and browning. Leaves from treated plants were usually 0-5% smaller in area than the non toxin-treated control leaves and after 3 d showed water-soaked infection centres typical of the susceptible response. To determine if HR suppression by phaseotoxin is accompanied by suppression of phytoalexin accumulation, an identical experiment was performed except that only one leaf sample was taken. Leaves of control and toxin-treated plants were collected after the appearance of HR in control plants (3 d after inoculation) and their phytoalexin content determined by thin-layer chromatography⁹.

Chromatograms of extracts from control tissues showed several prominent fluorescing bands when exposed to ultraviolet or when sprayed with diazotised *p*-nitroaniline. In extracts of the toxin-treated tissues, however, only faint bands were seen. The known phytoalexin bands were removed, eluted from silica gel with 95% EtOH and rechromatographed in chloroform-EtOH (100:3). The chromatographically pure compounds were quantitated by spectrophotometry. In control tissues, quantities of phaseollin, phaseollidin, phaseollinisoflaven and kievitone were 12.21, 7.67, 9.14 and 16.70 µg per g leaf tissue; whereas in toxin-treated tissues the quantities were 6.42, 3.04, 5.79 and 12.86 µg per g fresh weight of the tissue respectively.

The demonstration of suppression of phytoalexin accumulation in resistant bean tissues by phaseotoxin offers a mechanistic explanation for the suppression of HR in such tissues. That such suppression results at concentrations of toxin which are detected in infected susceptible tissues

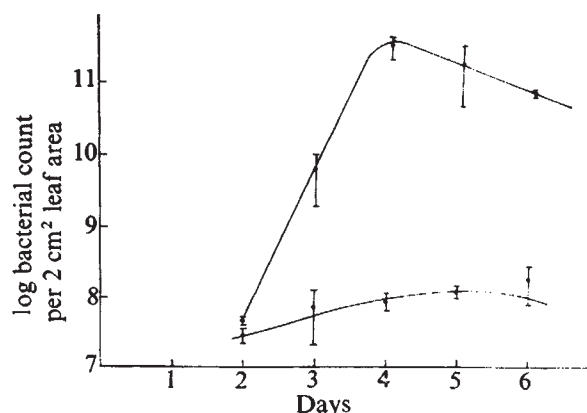


Fig. 1 Phaseotoxin treatment suppresses the resistance response in GN Nebraska 27 bean plants to *Pseudomonas phaseolicola*. Six-day-old seedlings were allowed to take up, through the roots, either quarter-strength nutrient solution or nutrient solution + phaseotoxin (4,200 U ml⁻¹) for 24 h in a growth chamber at 27 °C. Leaf disks were taken at the end of the 24-h period and their phaseotoxin level determined (1,300 U per 2 cm²). The toxin-treated (○) and control (●) plants were then replanted and their primary leaves infiltrated with a cell suspension (10^7 cells ml⁻¹) of *P. phaseolicola* and incubated in a growth chamber (16 h light, 24,000 lx) at 24 °C. Leaf disks (2.0 cm²) were removed daily from the leaves and their bacterial populations determined as described³. Bars represent s.e.m.

indicates that phaseotoxin is involved in the initial establishment of the pathogen in the susceptible tissues. That no phaseotoxin is detected in inoculated resistant tissues of bean³ further implicates the toxin in pathogenic establishment, and suggests that phaseotoxin production is suppressed in resistant tissues. Thus, it seems that the factor which determines bean resistance to *P. phaseolicola* has to do with suppression of elaboration of phaseotoxin.

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Collaboration between *in vivo* responses to LD and SD antigens of major histocompatibility complex

STUDIES using mixed leukocyte culture (MLC) and cell mediated lympholysis (CML) tests, have suggested a possible differential role for LD and SD antigens associated with the major histocompatibility complex (MHC, H-2 in mouse) in allograft reactions¹⁻³. LD differences are primarily responsible for stimulating the proliferative response in MLC; the SD antigens are the major targets in CML¹. The presence of LD differences on the stimulating cell in MLC enhances the cytotoxic response against the SD antigens. The cellular basis for this potentiation of CML is that two separate subpopulations of T lymphocytes are differentially reactive to LD and SD antigens^{1,3-5}. A proliferating helper cell responds primarily to LD differences whereas cytotoxic T lymphocytes are more strongly activated by the SD antigens. Lafferty *et al.*⁷ and Talmage *et al.*¹⁰ have shown that allogeneic thyroids transplanted under the kidney capsule are rejected less rapidly if they are first cultured *in vitro*. Injection of peritoneal exudate cells from the thyroid donor into the recipient speeds up rejection. They suggest that the peritoneal exudate cells sensitise the recipient and that the sensitised cells can invade and reject the thyroid. A further refinement in the interpretation of their data might suggest that either in addition to the mechanism they propose or as an alternative to it the peritoneal exudate cells provide an LD stimulus to the recipient's proliferating helper T cells, and that these cells either by themselves or through a secreted helper factor potentiate the development of cytotoxic T lymphocytes directed at the SD antigens on the graft. Our experiments reported here are consistent with this model.

The mouse strains we used are primarily strains AQR, B10.A and B10.T(6R). B10.A and B10.T(6R) are congenic on a B10 background. AQR carries a substantial portion of the B10 background but is not congenic with the other strains. Two mouse strains are said to be LD-different if they are identical for the two SD loci but differ for the

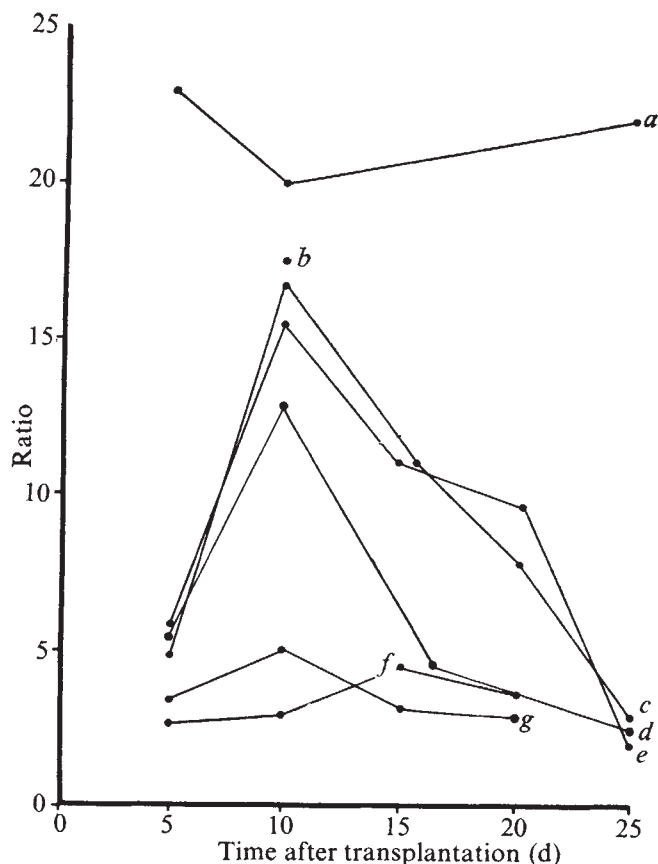


Fig. 1 Thyroid transplantation has been performed as described by Lafferty *et al.*⁶ and Talmage *et al.*¹⁰. Twenty-four hours before removal of the kidneys 0.5 μ Ci of ¹²⁵I was injected intraperitoneally. Both kidneys of each animal were counted in a gamma counter. The results are expressed as a ratio of the counts of ¹²⁵I in the thyroid-containing kidney to those in the contralateral control kidney. All thyroids were examined histologically for signs of rejection; the results are consistent with those expected based on the ratio. We have injected 1×10^4 lymph node cells (instead of peritoneal exudate cells) intraperitoneally into thyroid graft recipients. The H-2 complex can be divided into four regions, K, I, S and D. The SD loci are in the K and D regions; the strong LD locus is in I. The MHC genotypes of these strains are the following: AQR (qkdd), B10.A (kkdd) and 6R (qqdd), where the lower case letter in parentheses refers to the H-2 haplotype from which each region for a given strain is derived. Each point represents at least five animals. Comparative histological studies showed that thyroids with a ratio of 4 or lower reveal severe signs of rejection. The curves are keyed as follows: (recipient) (thyroid donor) (LNC donor). a, (AQR) (AQR) (none); b, (AQR) (AQR) (6R); c, (AQR) (B10.A) (AQR); d, (AQR) (B10.A) (B10.A); e, (AQR) (B10.A) (none); f, (AQR) (B10.A) (6R); g, (AQR) (B10.A) [(B10.A $\times$ 6R)(F₁)].

strong LD locus in the I region, and SD-different if a difference exists for either the K and/or D region but they are identical for the H-2 I region. Thus, AQR and 6R are LD-different, and AQR and B10.A are SD-different (being identical for the strong LD locus (loci)). We have used lymph node cells (LNCs) instead of peritoneal exudate cells.

The results are presented in Fig. 1. B10.A thyroids transplanted into AQR recipients with or without injection of AQR LNCs show prolonged survival compared with grafts differing for both H-2 LD and SD factors which in our experiments never show ratios above 4 (data not shown) tested on day 5 and later^{6,10}. Rejection is markedly more rapid when 6R LNCs are injected at the time of thyroid transplantation. Various controls are included in the figure. To rule out that the injected 6R cells themselves reject the thyroid rather than provide an LD stimulus to the recipient, in some experiments we have used [B10.A $\times$ (6R)] F₁ LNCs instead of the 6R LNCs; similar enhancement of rejection was seen. (AQR $\times$ B10) F₁ recipients gave results (not shown)

similar to the AOR recipients demonstrating that rejection is due to MHC differences (since AOR is not yet congenic with B10). Injection of B10.A LNCs into AQR recipients receiving a B10.A thyroid results in some acceleration of rejection. The potentiating effect is much less than that seen when 6R LNCs are injected and consistent with *in vitro* findings: B10.A stimulating cells not only induce a weak proliferative response in AQR responding cells, suggesting the presence of weak LD-like differences in the SD region of B10.A, but also lead to killing in CML⁷. Likewise, the fact that B10.A thyroids are rejected by AQR is consistent with these *in vitro* findings.

The most likely explanation of these findings is that LD-SD interaction similar to that seen *in vitro* can occur *in vivo*. When both an LD antigen on injected LNCs and SD differences on the B10.A thyroid are presented to the recipient's immune system, the B10.A grafts are rejected like LD+SD-different allografts. At the cellular level, this could be interpreted as follows. Injected LNCs from the LD-different 6R strain activate proliferating helper cells of the recipient. These cells, or soluble products secreted by them, amplify the response of the cytotoxic lymphocytes directed against the SD antigens in the thyroid.

B10.A thyroids are rejected in 20–25 d when no additional LNCs are injected. This delayed rejection is presumably due to the absence of strong LD-like differences in this combination. We suggest that the prolonged survival of allogeneic cultured thyroids, at least in part, is likewise due to the removal of the LD stimulus in the passenger leukocytes.

AQR recipients recognise H-2 I region associated antigens on the 6R LNCs; it could be argued that these antigens cross react with the SD antigens of B10.A recognised as foreign by AQR. *In vitro* data argue against this interpretation: AQR cells sensitised to 6R lyse 6R target cells but not B10.A^{1,7}. We thus consider this a very unlikely explanation for our findings and suggest instead that LD-SD collaboration occurs *in vivo* as well as *in vitro*.

Davies and collaborators^{8,9} have demonstrated that anti-I region (anti-Ia) antisera lead to prolongation of graft survival. Our studies suggest that, at least in part, I-regions disparity is important in allograft rejection in terms of activating the T-helper system; they thus provide a potential mechanism for explaining the effect of the enhancing anti-Ia antisera as well as extrapolating the *in vitro* findings of LD-SD collaboration to the *in vivo* system.

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Association of high affinity stereospecific binding of ³H-propranolol to cerebral membranes with β adrenoceptors

IN VITRO binding of ³H-catecholamines to plasma membranes from a variety of tissues has been reported^{1–4}. In none of these studies, however, has the binding satisfied criteria necessary for identification of true β adrenoceptors (for example, stereospecificity and high affinity for potent β -adrenoceptor antagonists). On the other hand studies using labelled β -adrenoceptor antagonists have demonstrated binding sites on erythrocyte ghosts and cardiac membranes that seem to satisfy fully the criteria associated with β adrenoceptors^{5–7}.

The presence of β adrenoceptors in cerebral tissue has been confirmed by electrophysiological techniques^{8,9}, and further characterisation of a central β adrenoceptor has come from biochemical approaches. For example, as is clearly the case in extracerebral tissue¹⁰, β adrenoceptors seem to mediate catecholamine-induced cyclic AMP formation in the brain^{11,13}. Catecholamines have also been shown to interact with a β adrenoceptor and markedly stimulate cyclic AMP formation in neonate chick cerebral hemispheres¹². Since it was felt that this tissue may represent a rich source of central β adrenoceptors, I have examined the characteristics of DL-³H-propranolol binding to membranes prepared from chick cerebral hemispheres.

Specific binding of DL-³H-propranolol to cerebral membrane fragments was saturable (Fig. 1) and half-maximal specific binding occurred at about 11 nM. On the other hand, nonspecific binding of DL-³H-propranolol (binding in the presence of 200 μ M isoprenaline) was not saturable over the concentration of propranolol examined. The amount of DL-³H-propranolol specifically bound at saturation provides an estimate of the number of binding sites on the membranes. The mean $\pm$ s.e. for five experiments was 0.23 ± 0.015 pmol per mg protein. DL-³H-propranolol bound rapidly and reversibly to cerebral membranes. Binding was 75% complete at 10 s and equilibrium was reached at 2 min, at which time the addition of unlabelled isoprenaline (200 μ M) resulted in a dissociation of bound DL-³H-propranolol with a half time of about 45 s.

The displacement of DL-³H-propranolol by non-radioactive stereoisomers, D- and L- and DL-propranolol is shown in Table 1. The D isomer was almost 100-fold less potent than the L isomer, and DL-pindolol proved to be the most potent β -adrenoceptor antagonist examined. The displacement of DL-³H-propranolol by catecholamines and salbutamol is also

Table 1 Effect of adrenergic antagonists and agonists on DL-³H-propranolol binding to crude synaptic membranes

Compound	Half-maximal inhibition of ³ H-propranolol binding
Antagonists	
L-Propranolol	9.5×10^{-9} M
DL-Propranolol	2.0×10^{-8} M
D-Propranolol	8.2×10^{-7} M
DL-Pindolol	6.1×10^{-9} M
DL-Pronethalol	1.4×10^{-6} M
Agonists	
L-Isoprenaline	1.2×10^{-6} M
L-Adrenaline	5.9×10^{-6} M
L-Noradrenaline	4.3×10^{-5} M
Salbutamol	1.1×10^{-5} M
Dopamine	No binding at 10^{-4} M

For binding studies DL-³H-propranolol (15 nM) was incubated with various concentrations of compounds shown and membrane protein equivalent to 0.6–0.7 mg for 15 min at room temperature. Results are means of triplicate determinations from four separate experiments using at least six different concentrations of drugs indicated; s.e.m. all < 10%.

shown in Table 1. Clearly the order of potency of the catecholamines was isoprenaline > adrenaline > noradrenaline. Salbutamol was about fourfold more potent than noradrenaline and dopamine was inactive at concentrations up to 100 μ M.

The results presented here demonstrate in a crude synaptic membrane preparation DL-³H-propranolol-binding sites that possess characteristics associated with true β adrenoceptors. Thus the binding is of high affinity, saturable, rapid, reversible and clearly stereospecific. Moreover, the cerebral membrane sites show high affinity for both β -adrenoceptor agonists and antagonists, and the order of potency of these compounds parallels their potency as agonists or antagonists on adenylate cyclase activity in various tissues^{5,7,15}. The potency of isoprenaline, adrenaline, noradrenaline and salbutamol in displacing DL-³H-propranolol binding from chick cerebral hemisphere membrane sites closely approxi-

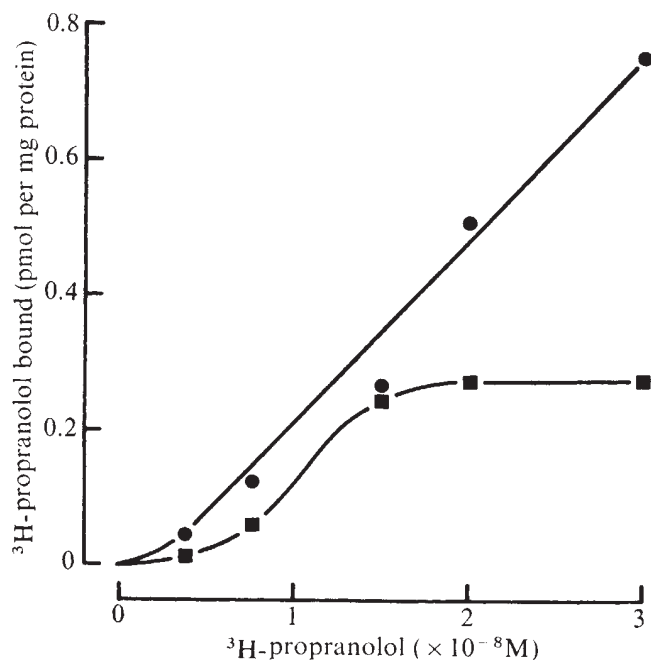


Fig. 1 Binding of DL-³H-propranolol to cerebral membranes. Male Ranger chicks (2–4 d old) were decapitated and their cerebral hemispheres homogenised in 20 volume ice-cold 0.32 M sucrose and the homogenate centrifuged at 1,000g for 10 min. Resulting supernatant was centrifuged at 17,000g for 30 min to obtain a crude mitochondrial pellet. This pellet (P₂) was lysed by re-suspending in 20 volumes ice-cold water and briefly rehomogenising. The suspension was centrifuged at 10,000g for 20 min and the resulting pellet, a bilayer with a soft buffy upper layer washed carefully to collect this upper layer. Supernatant was centrifuged at 48,000g for 20 min and final membrane pellets stored in liquid nitrogen until use. Protein estimations by the method of Lowry *et al.*¹⁴. In the binding assay, membrane suspensions (0.5–1 mg protein) were incubated in all experiments except where indicated, with 15 nM DL-4 μ -³H-propranolol (specific activity 21 Ci mmol⁻¹, Radiochemical Centre, Amersham), in buffer containing 50 mM Tris-HCl, pH 8.1, 15 mM MgCl₂ for 15 min at room temperature. Total volume of incubations 0.5 ml. Samples were filtered under pressure through Whatman glass-fibre circles (GFC) and immediately washed once with 10 ml buffer (filtering and washing required less than 6 s). Filters were dried and shaken in the cold with 5 ml Triton X100-toluene scintillator and radioactivity determined by liquid scintillation spectrometry. In every experiment 'nonspecific' binding was determined by measuring radioactivity obtained when incubations were carried out in presence of 200 μ M L-isoprenaline. Nonspecific DL-³H-propranolol binding (●) was subtracted from total radioactivity to obtain 'specific' binding (■). DL-³H-propranolol bound refers to 'specific' binding. At 15 nM DL-³H-propranolol, 'specific' binding was 40–50% total radioactivity. Results are means of triplicate determinations from five separate experiments; s.e.m. all < 10%.

mates the potencies of these compounds in stimulating cyclic AMP formation in slices of cerebral hemispheres of this species¹³.

The data reported in cerebral membranes agree with extensive studies examining DL-³H-propranolol and L-³H-alprenolol binding to erythrocyte and cardiac membranes^{5,6}, and suggests that the sites studied are indeed truly representative of β adrenoceptors. Earlier failures to detect stereospecific binding sites with DL-³H-propranolol in brain¹⁶ and heart¹⁷ probably relates to the use of high concentrations (100 nM) of DL-³H-propranolol rather than to the 'membrane' effects¹⁸ of this drug.

The use of high specific activity β -adrenoceptor antagonists should make it possible to quantitate and further characterise this cerebral receptor. In addition it should allow for a more definite cellular localisation of the β adrenoceptor since their presence on both neurones and glia has been suggested^{9,19}. Moreover, the use of DL-³H-propranolol should facilitate investigation of altered affinity and/or number of cerebral β adrenoceptors in conditions in which an apparent altered sensitivity to catecholamines has been demonstrated^{20,21}.

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Synaptic transmission reversibly conditioned by single-gene mutation in *Drosophila melanogaster*

ONE way to advance current physiological and biochemical understanding of the mechanism of synaptic transmission at the molecular level is to alter synaptic function by means of single gene mutations. Among the various behavioural mutants of *Drosophila melanogaster*, those which express a mutant phenotype only in certain conditions and otherwise behave normally are of particular interest. If the mutant phenotype appears at the synapse, the mutant might enable a reversible modification of synaptic function by way of experimentally controlling the expression of the

mutant phenotype. Such a mutant would provide a useful tool for the study of synaptic function.

Temperature-sensitive mutants which express their mutant phenotype only at a certain temperature are one class of conditional mutants. A single gene mutant of *D. melanogaster*, *shibire* temperature-sensitive (*shi^{ts}*), shows reversible paralysis at 29 °C, but behaves normally at 22 °C. Genetic and behavioural studies were previously done in Suzuki's laboratory¹⁻⁴. We report here that the paralysis results primarily from a blockade of neuromuscular transmission.

The material used was *shi^{ts1}*, one of the six alleles of the *shibire* locus. The mutant *shi^{ts1}* not only shows a reversible temperature-sensitive paralysis but also expresses morphological changes on the epidermal structure if it is exposed to high temperature during development². To avoid the latter effect, we have maintained the temperature during culture at 17 °C. Control wild-type Oregon-R flies were raised in the same conditions. Adult 4-d-old females of *shi^{ts1}* and Oregon-R were used. For the physiological experiments, a fly was mounted in wax and immediately covered with saline solution⁵ which was maintained at 19 °C by a thermoelectric unit placed under the bath. Artificial respiration was given through undamaged thoracic spiracles described previously⁶.

To confirm the temperature-sensitive paralysis, the mechanogram of the mesothoracic sterno-coxal muscle was recorded at various temperatures. For this purpose, the fly was placed ventral side up and the mesothoracic preepisternum was dissected out to expose the thoracic ganglion and the left mesothoracic leg nerve. The nerve was cut at about 20 μ m from the thoracic ganglion, and the distal cut end was sucked into a suction electrode for electrical stimulation. The left mesothoracic leg was cut at the coxal-trochanter joint, and a mechanoelectric transducer was attached to the proximal cut end of the leg. The other legs were held by wax at the femur to prevent disturbance to the record. The sterno-coxal muscle of *shi^{ts1}* responded to a single electric pulse with a vigorous twitch, as shown in Fig. 1a (*shi^{ts1}*) at 19 °C. When the temperature was increased to 27 °C, the magnitude of the response diminished. At 28 °C, only a small response was observed, and at 29 °C, the response was completely absent. The elimination of the mechanical response at 29 °C was

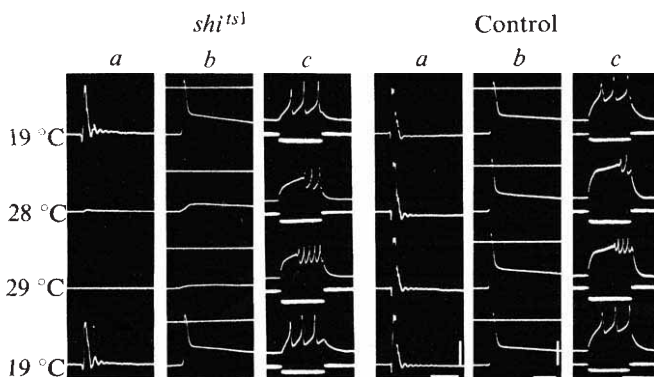


Fig. 1 Mechanical (a), junctional (b), and electrogenic (c) responses of mutant, *shi^{ts1}*, and control, Oregon-R, flies. a, Tension developed in mesothoracic sterno-coxal muscle on stimulation of mesothoracic leg nerve. b, Junctional and action potentials of third dorsal longitudinal muscle fibre in response to stimulation of posterior dorsal mesothoracic nerve. Upper trace in each record shows reference potential level. c, Muscle membrane responses of third dorsal longitudinal muscle fibre for transmembrane depolarising current. Upper trace: potential recording; lower trace: current recording, outward current downward. Temperatures are indicated at left of figures. Top 19 °C, beginning of experiment; bottom 19 °C, recovery after exposure to high temperature. Calibration for a: 2 mg, 250 ms; b: 50 mV, 10 ms; c: 50 mV, 1×10^{-7} A, 50 ms.

very specific and confirmed the previously reported behavioural results on paralysis³. When the temperature was lowered to 19 °C, the response returned almost completely. The change of temperatures from 19 to 29 and back to 19 °C was applied many times with consistently similar results, whereas the response from the control Oregon-R fly showed no appreciable change in mechanogram within this temperature range (Fig. 1). In several cases, the response of control flies remained unaffected even when the temperature was increased to 35 °C.

Our results indicated that the possible cause of paralysis is the excitability of the nerve, conduction of the impulse along the nerve, neuromuscular transmission, excitability of the muscle membrane, or contractile elements in the muscle fibre. From further studies, however, it seems that nerve excitability and conduction are not affected by the temperature change. To show this, the nerve was stimulated at the same location as described above, and nerve impulses were recorded at the femo-tibial joint as described previously⁵. The recorded nerve impulses from *shi^{ts1}* showed no appreciable diminution at 29 °C, nor did those from the control fly Oregon-R.

For further study of the neuromuscular properties of these mutants, we used the dorsal longitudinal flight muscle because a fibre of this muscle was known to be innervated by a single excitatory neurone⁷. For this purpose, a fly was mounted laterally and dissected as described previously⁷. The entire lateral surface of the dorsal longitudinal muscle and the thoracic ganglion was exposed to saline solution by making an opening at the lateral cuticle and removing the dorso-ventral and tergo-trochanter muscles. The posterior dorsal mesothoracic nerve innervating the exposed dorsal longitudinal muscle was cut about 50 μ m from the ganglion. The distal cut end was stimulated with a suction electrode. Intracellular recordings were made from the third muscle fibre. At 19 °C, both control Oregon-R and *shi^{ts1}* flies showed membrane potentials of -88 to -93 mV. The preparations showing resting potentials less negative than this range were discarded. In the remaining preparations, a single square current pulse of 0.1 ms was applied to the nerve every second, and the muscle membrane response was recorded continuously as the temperature was changed.

The muscle membrane response of *shi^{ts1}* is shown in Fig. 1b (*shi^{ts1}*). The top record of Fig. 1b (*shi^{ts1}*) shows the response at 19 °C. The response consisted of a rapidly increasing and overshooting action potential followed by the remaining slowly decreasing junction potential. When the temperature was raised, the action potential began to fail, leaving a junction potential of diminished size. At 28 °C only the junction potential was recorded. At 29 °C the junction potential was markedly reduced; and when the preparation was kept at 29 °C for longer than 5 min, no trace of junction potential was observed. Lowering the temperature to 19 °C restored the response completely. The effects of these changes of temperature were consistent and repeatable. During the change of temperature, the resting membrane potential showed no appreciable change and stayed at -90 mV. The response of the muscle membrane on stimulation of the nerve of the control fly Oregon-R is shown in Fig. 1b. The response at 19 °C was similar to that of *shi^{ts1}*. When the temperature was raised, the time course of the action potential became faster but never failed to respond even up to 35 °C. The results suggest that the possible cause of the paralysis in *shi^{ts1}* could be at the neuromuscular junction or more distal part of the system, possibly the muscle fibre membrane.

The excitability of the muscle membrane was tested by passing electric current through an intracellular microelectrode while observing the response by another intracellular electrode. As shown in Fig. 1c, the muscle membrane of both *shi^{ts1}* and Oregon-R flies responded with a train of

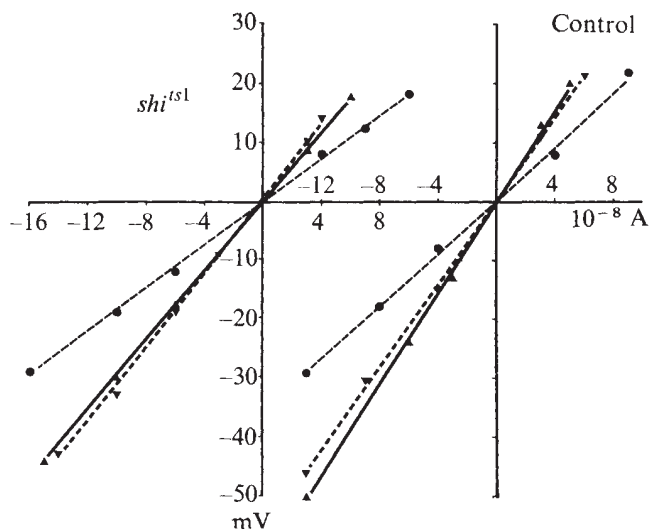


Fig. 2 Current-voltage relationship at different temperatures obtained from mutant, *shi^{ts1}*, and control, Oregon-R, flies. Δ , 19 °C, beginning; $\bullet$, 29 °C; ∇ , 19 °C, recovery. Depolarisation and outward current are plotted in positive direction on y and x axes, respectively. Resting potential located at origin.

action potentials after local depolarisation. At high temperatures, the amplitude of the action potential became smaller and a larger depolarising current was required to initiate action potentials. All of these effects of temperature, however, appeared similarly in both *shi^{ts1}* and Oregon-R flies. The current-voltage relationship of the muscle fibre membrane was analysed by applying hyperpolarising and depolarising currents to the fibre. As shown in Fig. 2, the input conductance increased by a factor of about 1.7 when the temperature was raised from 19 to 29 °C. The input conductance returned essentially to the original level when the temperature was lowered. These changes were common to both *shi^{ts1}* and Oregon-R flies. Thus, the capacity of electrogenesis of the muscle membrane was found to be unaffected by the *shi^{ts1}* gene.

So far, we have not investigated the effect of this mutation on the excitation-contraction coupling mechanism or the contractile protein. Therefore, we cannot eliminate the possibility that these and related properties necessary for the contraction of the muscle are affected by mutation. If the neural information carried by the nerve fails to be transmitted, however, to the muscle membrane at the neuromuscular junction, as shown by *shi^{ts1}* at high temperature, the failure of muscle contraction is independent of the state of the contractile mechanism. Thus, the major cause of paralysis induced by the *shi^{ts1}* gene is the blockade of neuromuscular transmission.

We have not determined whether the failure of transmission is presynaptic or postsynaptic. Kelly and Suzuki⁸, however, reported that the loss of the on-off transient of electroretinogram of *shi^{ts}* at high temperature was attributable to both pre- and postsynaptic defects. It is likely that not only the neuromuscular junction but other synapses are affected by the *shi^{ts}* gene. At the presynaptic terminal, if the potential-dependent calcium permeability⁹ is affected, the transmitter release mechanism may be altered. The action potential of the muscle fibre is produced by the potential-dependent increase in calcium permeability (unpublished data on *Drosophila* by the present authors and also on *Sarcophaga* by J. B. Patlak). Note that the calcium permeability of the muscle fibre membrane is not affected in *shi^{ts1}*.

At the postsynaptic membrane, one possibility is the direct effect on the receptor protein; it is known that the change of a single amino acid in a polypeptide could result in altering the temperature sensitivity of the protein¹⁰.

Membrane permeability changes at the subsynaptic membrane also should be investigated. Although we have not determined these points, the present results are the first to reveal that a temperature-conditioned neuromuscular transmission can be induced by a single gene mutation. Siddiqi and Benzer reported similar results (unpublished) with another allele of *shi^{ts}*.

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Ca²⁺ influx across the excitable membrane of behavioural mutants of *Paramecium*

THE ciliate protozoan *Paramecium* has an excitable surface membrane, which depolarises in response to a sudden increase in the Na⁺ or K⁺ concentration of the medium. An influx of Ca²⁺ during depolarisation raises the internal Ca²⁺ concentration, which in turn causes the cilia to reverse their direction of beating, and the cells to swim backwards¹. The swimming behaviour of *Paramecium* is therefore a direct and readily observable correlate of the electrophysiological state of the membrane.

We have developed (unpublished results) a method of measuring ⁴⁵Ca²⁺ influx and shown that in wild-type cells, the rate of ⁴⁵Ca²⁺ influx increased five- to tenfold when 10 mM Na⁺ or K⁺ was added. This stimulated influx correlated with the observed backward swimming which in turn is correlated with Ca²⁺ movement down an electrochemical gradient through a Ca²⁺ "gate". This gating mechanism is a major component of membrane excitability in *Paramecium*^{1,2}.

By selecting for behavioural defects, Kung *et al.* isolated several classes of mutant paramedia defective in various components of membrane excitability². We have measured ⁴⁵Ca²⁺ influx in all the major classes of mutants. Cells that showed no backward movement in Na⁺ or K⁺, also showed little Na⁺- or K⁺-stimulated Ca²⁺ influx.

Ca²⁺ influx was measured essentially as before (to be described elsewhere). A low Ca²⁺ concentration was used to minimise Ca²⁺ binding, and the assay was performed at 0 °C to inhibit an active Ca²⁺ extrusion. Cells were separated from the assay solution by centrifugation in a Pasteur pipette with one end sealed³.

Both behaviour and cell survival at 0 °C were examined. In the assay solution (legend, Table 1) at 0 °C, the cells

Table 1 Rate of Ca^{2+} influx in behavioural mutants of *Paramecium*

Phenotype	Genotype and strain	Growth temp. ($^{\circ}\text{C}$)	Rate of Ca^{2+} influx				Backward swimming in	
			Control	NaCl (mM)		KCl (mM)		Na ⁺ K ⁺
Wild type	(51s)	24-26	3.0	8.5	11.0	12.3	16.0	+ ++
		24-26	1.7	6.2	11.5	15.0	19.0	
		24-26	1.1	1.2	4.0	6.6	13.4	
		35	3.0	8.0	9.2	11.0	10.5	+ ++
		35	1.2	4.3	4.5	4.5	6.6	
Pawn	<i>pwa</i> (d4-94)	24-26	1.1	1.7	2.5	1.1	3.3	— —
		24-26	2.0	2.7	2.8	2.1	5.4	
	<i>ts-pwa</i> ² (d4-133)	24-26	1.0	6.1	7.6	11.0	16.3	+ ++
		24-26	2.6	6.5	6.3	7.4	11.1	
		35	2.3	1.7	2.1	2.6	2.6	— —
		35	0.8	0.7	1.0	1.1	0.6	
	<i>pwb</i> (d4-95)	24-26	1.2	1.5	2.0	0.8	1.4	— —
		24-26	1.1	1.0	1.0	1.0	1.0	
	<i>ts-pwC</i> (d4-131)	24-26	1.1	2.2	2.1	3.7	5.9	+ ++
		24-26	1.3	2.6	3.0	2.5	4.0	
		35	2.3	1.3	1.7	1.7	2.5	— —
		35	1.0	0.5	1.0	1.0	1.2	
Paranoiac	<i>PaA</i> (d4-90)	24-26	2.7	19.3	16.8	18.7	15.2	++ ++
		24-26	1.7	9.2	8.9	9.0	9.0	
	<i>PaC</i> (d4-150)	24-26	1.7	8.8	12.4	12.5	9.3	++ ++
		24-26	1.0	13.0	13.5	7.0	7.2	
	<i>fna</i> ^P (d4-149)	24-26	1.5	3.5	4.0	4.2	7.5	++ ++
		24-26	1.5	3.5	4.0	5.5	13.0	
Fast-1	<i>fA</i> (d4-98)	24-26	2.4	7.7	5.7	7.7	8.7	+ ++
		24-26	2.6	5.8	5.6	8.2	11.3	
Fast-2	<i>fna</i> (d4-91)	24-26	2.0	2.2	1.7	10.5	10.1	— ++
		24-26	2.1	2.0	2.0	12.4	13.5	
TEA ⁻	<i>TEA</i> (d4-152)	24-26	2.0	7.0	8.5	6.0	9.0	+ ++
		24-26	1.2	3.2	3.6	7.0	9.0	

Influx was measured as nmol Ca^{2+} per 10 min per mg protein. Mutants were derived from 51s and were homozygous for mutations at the designated gene loci. Strain numbers are given in parentheses. —, No backward swimming observed; +, cells show repeated backward jerks; ++, cells swam backwards continuously.

Paramecium aurelia (syngen 4) was grown in cerophyl medium⁷ (supplemented with β -sitosterol (1 mg l⁻¹) and inoculated 16 h previously with *Enterobacter cloacae*, the food source). Cells were collected by centrifugation at mid to late logarithmic phase, washed once in the assay solution and transferred into the assay solution. The assay solution consisted of: 10% v/v cerophyl medium (prepared without Na_2HPO_4), 1 mM HEPES 50 μM CaCl_2 , 20 μM EDTA and Tris base to pH 7.0. This solution contained 40 μM free Ca^{2+} . Cells (30–90,000 per ml) were left in the assay solution for 30 min at room temperature. Then, the cells were placed on ice for 20 min before $^{45}\text{Ca}^{2+}$ influx was measured and were left on ice for the rest of the experiment. $^{45}\text{CaCl}_2$ (New England Nuclear, titrated to a phenol red endpoint with Tris base) was added to a specific activity of Ca^{2+} of 1,000 c.p.m. nmol⁻¹. Duplicate samples of cells were collected by layering 0.5 ml of the cell suspension over 1.0 ml of wash solution (assay solution with 1% sucrose), in a chilled, siliconised Pasteur pipette centrifuge tube. The tube was centrifuged for 0.5–1.0 min at 500g in an oil testing centrifuge and the tip containing the cell pellet was broken off. The tips were crushed and counted by liquid scintillation in a Triton-toluene mixture⁸. Uptake was approximately linear over the first 20 min. Rates were determined at 10 and 20 min and the average was taken. A large amount (30–50%) of the total Ca^{2+} in the assay mixture had been taken up after 30 min at 0 $^{\circ}\text{C}$ when cells were stimulated by 10–15 mM KCl or NaCl. When the number of cells in the assay was decreased, however, we found no appreciable difference in uptake rates, expressed as nmol Ca^{2+} per 10 min per mg protein.

swam, though more slowly than at 25 $^{\circ}\text{C}$, for at least 1 h. When Na^+ or K^+ (10–15 mM) was added, 20–80% of the cells swam very slowly or stopped after 10–30 min. These cells swelled slightly, yet lysis was never observed. Naitoh⁴ has shown that cilia cease to beat when the intracellular Ca^{2+} concentration rises above 0.1 mM. Cells stopped swimming at the end of the experiment only when Na^+ or K^+ was added, at a final concentration of 10 mM or higher. Pawn mutants, which did not show stimulated Ca^{2+} influx, also did not stop swimming in the presence of Na^+ or K^+ . For these reasons, we believe that the poor condition of the cells at the end of the experiment is a result of a large accumulation of intracellular Ca^{2+} in our assay conditions, which were chosen to prevent normal Ca^{2+} expulsion. The increased intracellular Ca^{2+} concentration apparently affects cell motility and shape.

Wild-type cells at 0 $^{\circ}\text{C}$ swam backwards or showed repeated backward jerks in solutions containing Na^+ or K^+ , as they did at room temperature. The response of mutant cells to Na^+ and K^+ at 0 $^{\circ}\text{C}$ was also qualitatively the same

as the response at room temperature. In the absence of Na^+ or K^+ , spontaneous avoiding reactions were more frequent at 0 $^{\circ}\text{C}$ than at room temperature. These spontaneous reversals were probably due to an abnormal accumulation of Ca^{2+} , which occurred when the Ca^{2+} efflux system was inhibited by low temperature.

Ca^{2+} influx in wild-type cells was stimulated by both Na^+ and K^+ , with K^+ more effective than Na^+ (Table 1). This trend was observed in each of approximately 20 experiments with wild-type cells. The absolute rate of stimulated Ca^{2+} influx varied over a range of 6–20 nmol per mg protein per 10 min in different experiments. This variability may be due to differences in the nutritional state of the cells which are difficult to control. In starved or slowly growing cells, Na^+ generally stimulated Ca^{2+} influx only weakly.

Ca^{2+} influxes were measured for 10 behavioural mutants of *Paramecium*, each with a different, single gene mutation. These mutants belong to the following classes: pawns, paranoiacs, fast-1, fast-2, and TEA (tetraethyl ammonium)-insensitive.

Pawn mutants always swim forward and do not reverse their swimming direction in Na^+ or K^+ . This behaviour has been attributed to a mutation in a voltage-sensitive Ca^{2+} gate, which cannot open properly to allow Ca^{2+} down its electrochemical gradient². Na^+ and K^+ failed to stimulate Ca^{2+} influx in the unconditional pawn, *pwB*, and in the temperature-sensitive pawns, *pwA*², and *pwC* grown at 35 °C (Table 1). Ca^{2+} influx in the pawn mutant *PwA* was weakly stimulated by Na^+ and K^+ . This mutant is somewhat leaky

(C. Kung, personal communication). Temperature-sensitive pawns exhibit wild-type behaviour when grown at room temperature and pawn behaviour when grown at 35 °C (ref. 6). Figure 1 shows the effects of Na^+ and K^+ on Ca^{2+} influx in wild-type and temperature-sensitive pawns grown at 24–26 °C and at 35 °C. Temperature-sensitive pawns require 2–4 h of growth at the permissive temperature to shift from pawn to wild-type behaviour³, so cells grown at 35 °C could not have shifted into a new phenotype during the assay at 0 °C.

The pawn mutant *pwC* (d4-131) has reproducibly lower rates of Ca^{2+} influx when grown at the permissive temperature (24–26 °C) (Table 1) and may therefore be somewhat abnormal even when grown at the permissive temperature. Kung has presented genetic evidence that one or both of the gene products of *pwA*¹ and *pwC* do not function normally even at the permissive temperature. Although both *pwC* and *pwA*¹ are temperature-sensitive pawns, the double mutant *pwA*¹/*pwA*¹ *pwC*/*pwC* is temperature independent^{5,6}.

Paranoiac mutants swim backwards for abnormally long periods in Na^+ , yet swim backwards normally in K^+ (ref. 2). The biochemical nature of the defect is not known. Na^+ was as effective as K^+ in triggering Ca^{2+} influx in the paranoiac *PaA*; and in another paranoiac strain, *PaC*, Na^+ stimulated Ca^{2+} influx even more effectively than K^+ (Fig. 1 and Table 1). The rates of Ca^{2+} influx in the paranoiacs were correlated with the behaviour of paranoiacs, since Na^+ -stimulation caused increases in both the duration of backward swimming and the rate of Ca^{2+} influx.

Fast-1 and fast-2 mutants swim faster than normal cells. Fast-1 responds normally to Na^+ and K^+ , while fast-2 reverses only in K^+ but not in Na^+ (ref. 2). Fast-2 cells seem to have an abnormally large resting K^+ permeability (Y. Satow, and C. Kung, personal communication). Ca^{2+} influx in fast-1 cells was stimulated normally by Na^+ and K^+ , while only K^+ stimulated Ca^{2+} influx in fast-2 (Table 1 and Fig. 1). Again, there was a direct correlation between Ca^{2+} influx and ciliary reversal.

The mutant *fna*^p behaves as a paranoiac, but the mutation is located in the fast-2 gene (*fna*) (J. Van Houten, S-Y. Chang, and C. Kung, personal communication). Na^+ stimulated Ca^{2+} influx in this cell line to a rate similar to that in wild type (that is, intermediate between fast-2 and paranoiac). K^+ -stimulated Ca^{2+} influx normally in the *fna*^p mutant (Table 1).

The mutant *TEA*⁻ swims forward instead of reversing in the presence of TEA (which causes backward jerking in wild-type cells). The *TEA*⁻ mutant responds almost normally to Na^+ and K^+ (ref. 2), but it apparently has a membrane defect resulting in a low resting resistance, most likely due to an increase in conductance to potassium (Y. Katow, and C. Kung, personal communication). *TEA*⁻ cells had normal Na^+ - and K^+ -stimulated Ca^{2+} influx (Table 1).

There was an excellent correlation for all the cell lines studied between an increased Ca^{2+} influx triggered by Na^+ and K^+ and backward swimming stimulated by Na^+ and K^+ . These results further document that the three pawn genes, *pwA*, *pwB* and *pwC* are involved with the Ca^{2+} "gate", since these are the only mutants that show little Ca^{2+} influx in both Na^+ and K^+ solution. Our results suggest that fast-2 and paranoiac mutants, which show altered behaviour in Na^+ , have normal Ca^{2+} "gates", since they show normal Ca^{2+} influxes in K^+ solution. The abnormal response to Na^+ in these strains is apparently the result of other factors. The variation between wild type, paranoiac and fast-2 phenotypes could be explained by postulating separate Na^+ - and K^+ -triggered Ca^{2+} "gates". We consider separate gates unlikely, since a single mutation in pawns can eliminate all Ca^{2+} -mediated depolarisations².

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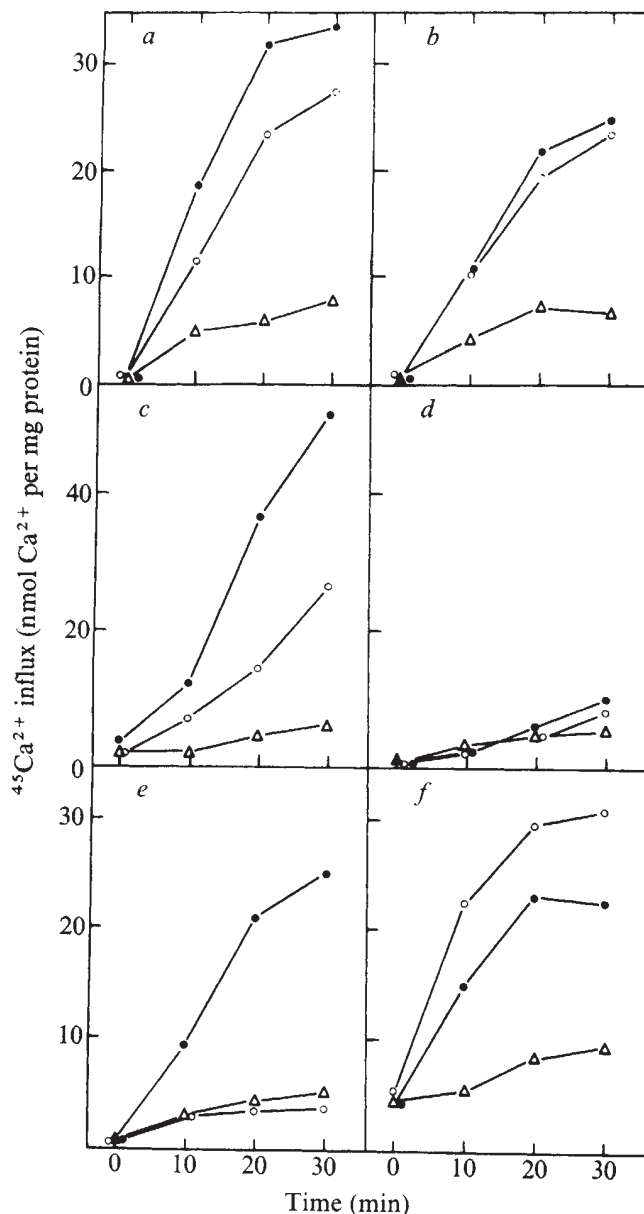


Fig. 1 $^{45}\text{Ca}^{2+}$ influx in wild type and behavioural mutants of *Paramecium*. Ca^{2+} influx was assayed as described in the legend to Table 1. Cell cultures were grown at 24–26 °C as described in the legend to Table 1. Cells were fed with fresh cerophyl medium which had been inoculated 12 h previously with *Enterobacter cloacae*. Half of the culture was left at 24–26 °C and the other placed at 35 °C. Ca^{2+} influx was assayed after 20 h or more. Each point represents the average of duplicate samples. The difference between duplicate values averaged 0.7 nmol Ca^{2+} per mg protein, for 40 separate points. Ca^{2+} influx was measured with 15 mM KCl (●), 15 mM NaCl (○), and the control (no addition) (△). a, wild type (25 °C); b, wild type (35 °C); c, *ts-pwA*² (d4-133, 25 °C); d, *ts-pwA*² (d4-133, 35 °C); e, *fna* (d4-91, 25 °C); f, *PaC* (d4-150, 25 °C).

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Synthesis of ATP coupled with action of membrane protonic pumps at the octane-water interface

ATP SYNTHESIS in mitochondria is one of the most important energy-producing processes in the cell. We have studied the main stage of this process, the coupling of ATP synthetase to the action of membrane protonic pumps at an octane-water interface.

Two hypotheses exist concerning this coupling of respiration to phosphorylation. That of Williams¹ suggests direct proton transfer in lipid phase from respiration enzymes to ATP synthetase, whereas that of Mitchell² postulates an essential additional stage of transmembrane proton transfer, resulting in the formation of a membrane potential. We have carried out ATP synthesis in non-equilibrium conditions (acidic concentration in the hydrophobic phase), thus supporting William's hypothesis.

We had previously shown that the mitochondrial ATPase can transfer protons from water to octane by ATP hydrolysis³. We therefore carried out the reverse reaction, and observed ATP synthesis coupled to the action of purified mitochondrial ATPase adsorbed at an octane-water interface. The proton flow through the ATPase complex from octane to water was caused by an excess concentration (with respect to that of the equilibrium) of the undissociated acid or Lewis form in the octane phase.

The excess concentration of acid was produced by oxidation of the reduced form of nicotinamide adenine dinucleotide (NADH) by ferricyanide with the participation of the enzymes comprising the initial step in the respiratory chain of mitochondria. For this purpose, submitochondrial particles (SMP) (for isolation techniques, see refs 4 and 6), were introduced into the octane-water system, to which was added one of the ATPases to be examined and rotenone, an inhibitor of NADH oxidase which prevents respiration and thus the synthesis of ATP in SMP. If NADH is added to the octane-water system containing a proton acceptor 2,4-dinitrophenol (DNP), $K_3Fe(CN)_6$, SMP and rotenone, the maximum shift in the positive direction of the potential difference measured in the chain, Au-air-octane, proton acceptor (RO^- , ROH)-water, enzyme, substrate-water, saturated $KCl-Hg_2Cl_2/Hg$, was 0.52 V. ATP synthesis at the interface was detected by a decrease in potential difference measured in this chain on addition of ADP and inorganic phosphate to the system. Equilibrium octane-water systems containing 50 mM Tris-HCl (1 mM) and DNP were used.

Enzyme and substrates were added just before measuring the potential differences by the dynamic capacitance method^{7,8-10}. Voltages plotted in the figures are referred to the initial value.

When 0.2 mM NADH was added to the system containing SMP, $K_3Fe(CN)_6$ and oligomycin-sensitive ATPase, a potential shift in the positive direction was observed at the octane-water interface. Subsequent addition of 1 mM ADP and 1 mM inorganic phosphate caused the potential difference to shift in the negative direction. At sufficiently high ADP concentrations this negative shift was below the initial value (Fig. 1). Addition of oligomycin (points C and C', Fig. 1) eliminated almost completely the action of ADP and phosphate on the potential difference in the oligomycin-sensitive ATPase system.

Instead of SMP, another proton pump, such as bacteriorhodopsin sheets (obtained from *Halobacterium halobium*), was used. As we have shown previously⁸, bacteriorhodopsin sheets are also capable of transferring protons from water to octane by the action of light. Figure 2 shows that when light is switched on, bacteriorhodopsin sheets cause a potential change (0.5 V) at the octane-water interface. When the ATPase reaction starts, however, the potential returns to its initial value. When oligomycin is added in the presence of oligomycin-sensitive ATPase of *p-N,N*-di(2-chloroethyl)aminophenylacetic acid, an alkylating agent and inhibitor of soluble mitochondrial ATPase, bacteriorhodopsin sheets again generate the photopotential. Figure 2 shows the change in potential difference in the above chain in the presence of bacteriorhodopsin sheets on illumination and in the dark, as affected by the concentration of

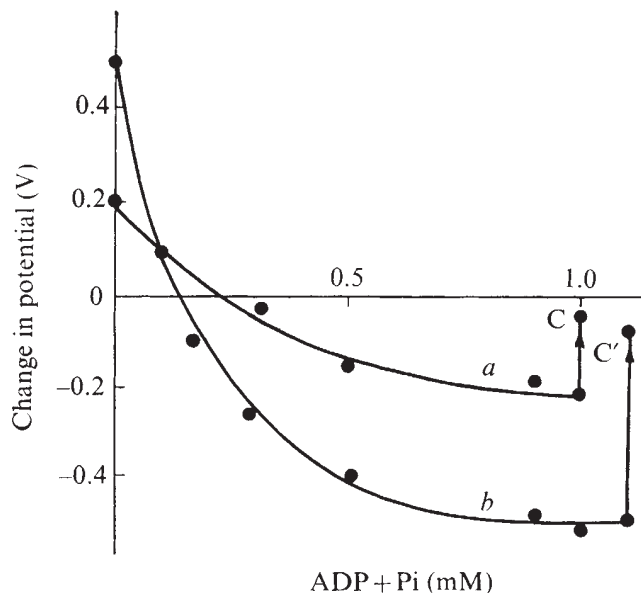


Fig. 1 Shift in negative direction of potential difference in the chain (see text) as affected by the concentration of substrate ADP+Pi. Composition of incubation medium: *a* 0.1 mg ml⁻¹ oligomycin-sensitive ATPase, 0.1 mg ml⁻¹ SMP (isolated by the technique used in ref. 4), 2.5 µg ml⁻¹ rotenone, 50 mM Tris-HCl, 1 mM DNP, 1 mM MgSO₄, 1 mM K₃Fe(CN)₆ and 0.2 mM NADH. *b*, As *a* except SMP was isolated by the technique used in ref. 2. At points C and C' 2 µg ml⁻¹ oligomycin was added. The following experiments were run as controls: (1) Oligomycin-sensitive ATPase was excluded from the complete system. In these conditions, addition of ADP and Pi does not affect the potential difference. (2) SMP was excluded from the complete system. Addition of ADP and inorganic phosphate does not affect the potential difference. (3) NADH is excluded from the complete system. A very small potential change (no more than 25 mV) was observed on addition of 1 mM ADP and 1 mM Pi. Inorganic phosphate was excluded from the complete system. A very small potential change (no more than 25 mV) was observed on addition of 1 mM ADP.

Table 1 ATP synthesis at the octane-water interface due to the excess concentration of PCP in octane

ATPase	Experimental conditions	Amount of ATP in sample ($\times 10^{-6}$ M)	Mean amount of ATP synthesised ($\times 10^{-6}$ M)
Oligomycin-sensitive ATPase ($100 \mu\text{g ml}^{-1}$) + ADP (10^{-4} M)	-PCB +PCB +PCB+oligomycin (1 g ml^{-1})	3.1 4.2 2.9	— 1.1 —
Soluble mitochondrial ATPase before heat treatment ($130 \mu\text{g ml}^{-1}$) + ADP (3.10^{-5} M)	-PCB +PCB	2.5 3.7	— 1.1

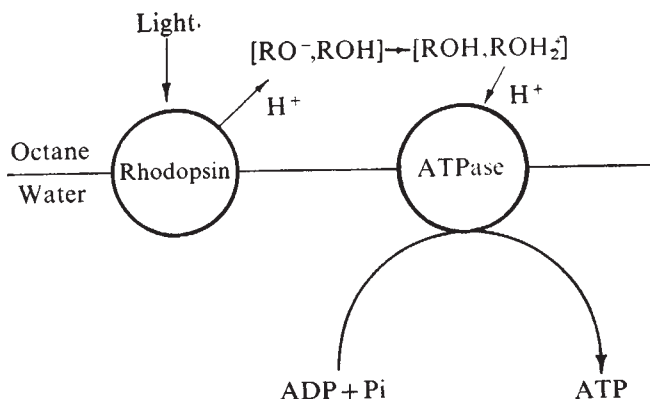
the substrate ADP and inorganic phosphate. When ADP and inorganic phosphate are added to a system with an active proton pump, the potential rapidly decreases.

ATP synthesis by oligomycin-sensitive ATPase, adsorbed on the octane-water interface, also takes place after addition of pentachlorophenol-octane solution to the octane phase. The system contained 1 ml octane and 2 ml 20 mM phosphate buffer ($\text{pH}=7.5$) to which were added 40 g hexokinase protein, 10^{-4} M ADP, 20 mM glucose, 2 mM MgSO_4 , and $100 \mu\text{g}$ protein of oligomycin-sensitive ATPase isolated from the mitochondria of ox heart by Racker's method². After introduction of 0.02 ml PCP into octane, its concentration in the complete system was 5×10^{-5} M. After 1 min the reaction was stopped by perchloric acid and neutralised with NaOH. The amount of ATP formed was determined fluorimetrically by the glucose-6-phosphate dehydrogenase method. In the control tests PCP and octane were excluded from the system. The results of these tests (Table 1) show that in the control tests ATP is present as an impurity in the reactants used and can be formed according to the equation $2\text{ADP} \rightarrow \text{ATP} + \text{AMP}$, because of the presence of myokinases in the enzymes used. In the complete system the amount of ATP formed was 30–50% higher than in control tests in which PCP was not added. The experimental error was no greater than 10%. In the experiments with oligomycin-sensitive ATPase, oligomycin lowered the ATP level to the control value.

Figure 3 shows a scheme for the action of ATPase at the interface, based on the experimental data described above. It is clear from this scheme that under the action of light, in the presence in the non-aqueous phase of a proton acceptor (such as a weak acid, ROH), bacteriorhodopsin transfers protons to this phase. Of course, protons do not exist in the free form in the non-aqueous phase, but are

captured by RO^- or ROH to form ROH or ROH_2^+ (Lewis acid form¹⁹).

If the charged Lewis acid form alone acted in this process as proton donor, the potential would always be positive and equal to, or less than, the initial positive charge of octane obtained in the case of SMP (Fig. 1). The change in the potential sign in the negative direction observed in the experiment with SMP must mean that ATP synthesis is also brought about by the elimination of protons from the neutral ROH form of DNP. In this case the anion remains in the non-aqueous phase and the proton goes into water. To verify this assumption, we used dimethoxybenzene

**Fig. 3** Scheme for ATP synthesis at octane-water interface brought about by action of bacteriorhodopsin sheets.

instead of DNP as Lewis-type proton acceptor in the non-aqueous phase. In this case, when NADH is added to the system containing soluble mitochondrial ATPase and SMP, the potential shifts in the positive direction by 0.2 V. At high ADP and inorganic phosphate concentrations it does not shift in the negative direction.

The interpretation of our experimental data obtained from measurements of the potential difference in the above chain is being verified by direct determinations of the amount of ATP synthesised. Our data show that the presence of a transmembrane potential is not a necessary condition for ATP synthesis by ATPase. The energy of proton (acid) solvation can be used for ATP synthesis.

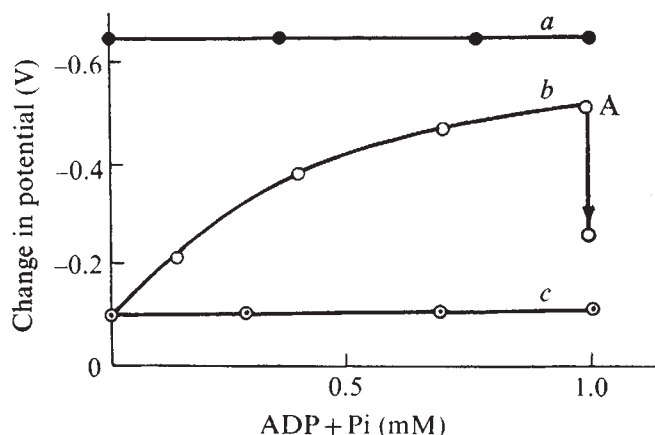
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Fig. 2 Dependence of potential difference measured in the chain (see text) on the concentration of substrate ADP + Pi. Composition of incubation medium: a, $30 \mu\text{g ml}^{-1}$ bacteriorhodopsin sheets, 1 mM DNP, 50 mM Tris-HCl and 1 mM MgSO_4 in the dark; b, as c plus 10^{-8} M soluble mitochondrial ATPase; c, as a, b and c under illumination. $\text{pH} = 7.9$. At A, 1 mM *p*-N,N-di-(2-chloroethyl)aminophenylacetic acid has been introduced into the cell

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Antiviral, immunosuppressive and antitumour effects of Ribavirin

THE compound 1- β -D-ribofuranosyl-2,2,4-triazole-3-carboxamide (Ribavirin) has been reported to inhibit the replication of both RNA and DNA viruses *in vitro*, and to inhibit influenza virus infection of tissue cultures and mice^{1–3}. The compound acts by interfering with guanine monophosphate formation and subsequent nucleic acid synthesis; at a concentration which completely inhibited influenza viral polypeptide production in tissue culture, however, there was no demonstrable effect on cellular protein synthesis⁴. To test the activity of Ribavirin further, we examined the effect of this compound on influenza virus

Table 1 Response of ferrets to infection with influenza virus A/Port Chalmers/73

	Control ferrets	Ferrets treated with Ribavirin (100 mg kg ⁻¹ d ⁻¹)
Response to infection*		
Rise in temperature† (°C)	39.3–40.9	39.6–39.6
Virus titre in nasal wash at 48 h post-infection (EID ₅₀ ml ⁻¹)	10 ^{6.30}	10 ^{4.35}
Nasal wash protein concentration; pre- and 7 d post-infection (mg per 100 ml)	0.28–1.22	0.26–0.43
Nasal wash neutralising antibody titre; pre- and 7 d post-infection	<2–1:14	<2–<2
Serum HI antibody response (g.m.t.)	<5–463	<5–<5

*Mean values for four ferrets.

†Increase in temperature from mean, pre-inoculation temperature to temperature at 48 h post-infection.

infection of ferrets. Four adult ferrets were inoculated intraperitoneally with 100 mg kg⁻¹ d⁻¹ of Ribavirin each for 7 d; this dose although it did not cause deaths or other signs of toxicity, was probably close to the toxic level as judged by standard tests in mice. Two hours after the second inoculation of drug, these animals and four

untreated ferrets were lightly anaesthetised and inoculated intranasally with 10^{3.0} ferret-infective doses of egg-grown influenza virus A/Port Chalmers/73 (H3N2). Following virus infection, the drug-treated ferrets were given 5 further doses of Ribavirin. The response of the animals to virus infection was measured, as described previously^{5,6}. The results are shown in Table 1. Ribavirin had a marked effect on the response of ferrets to influenza virus infection; compared with control ferrets, influenza virus-infected animals treated with Ribavirin did not exhibit a febrile reaction to infection, did not develop a significant increase in nasal wash protein and did not develop either local or serum antibody. In addition, the titre of virus in nasal washings collected 3 d after virus infection from drug-treated animals was 100-fold less than that found in control ferrets.

The absence of both a serum and a local antibody response in Ribavirin-treated ferrets infected with influenza virus may have been due to the antiviral activity of the compound which reduced virus replication to a level that did not induce antibody production (Table 1). Alternatively, Ribavirin may also have an immunosuppressive effect. To test this latter possibility, a group of guinea pigs was inoculated intraperitoneally with 100 mg kg⁻¹ d⁻¹ of Ribavirin for 10 d. On the second day, the drug-treated animals and a group of untreated guinea pigs were inoculated intramuscularly with 400 IU of inactivated influenza virus A/England/42/72 (H3N2) vaccine in an equal volume of Freund's complete adjuvant (FCA). Serum samples were collected before and 10 d after immunisation, and tested for serum haemagglutination inhibiting (HI) antibody. Some guinea pigs in each group were inoculated intraperitoneally with 10 ml of sterile paraffin 4 d after immunisation and were killed 6 d later; the peritoneal macrophages were collected from these animals and tested for cell-mediated immunity to influenza virus by the macrophage migration inhibition test. Ten days after immunisation, peripheral blood specimens were collected from the guinea pigs, and the buffy coat cells incubated with 2.0 μ g ml⁻¹ of phytohaemagglutinin and tested for lymphocyte transformation. The results are shown in Table 2. Guinea pigs immunised with influenza virus vaccine in FCA produced serum HI antibody by 10 d, post-inoculation (geometric mean titre g.m.t.=1:124); at this time however, no antibody was detectable in serum from Ribavirin-treated animals. The migration of peritoneal macrophages from both control and drug-treated guinea pigs was significantly inhibited in the presence of 40 IU of influenza virus A/England/42/72 vaccine. Furthermore, lymphocytes from drug-treated and control animals were both transformed by PHA to approximately the same degree (Table 2). The results indicate that Ribavirin, at

Table 2 Immune response of guinea pigs to inoculation with 400 IU of inactivated influenza virus A/England/42/72 vaccine in FCA

Ribavirin treatment	Animal no.	Serum HI antibody response (g.m.t.)		Delayed hypersensitivity response (MMI test) to 40 IU influenza vaccine at day 10, post-infection			Response to PHA (2.0 μ g ml ⁻¹) at day 10, post-inoculation		
		day -1	day 10	Test $\pm$ s.d.*	Control $\pm$ s.d.	Inhibition %	Test (c.p.m. $\times 10^{-4}$)	Control (c.p.m. $\times 10^{-4}$)	Fold stimulation
100 mg kg ⁻¹ d ⁻¹ from day -1 to 10	1	<5	<5	NT	NT	NT	22.2	2.1	10.7
	2	<5	<5	0.25 $\pm$ 0.01	0.48 $\pm$ 0.04	54†	7.9	1.3	6.1
	3	<5	<5	0.66 $\pm$ 0.02	1.25 $\pm$ 0.11	47†	NT	NT	NT
	4	<5	<5	NT	NT	NT	NT	NT	NT
Nil	5	<5	512	0.75 $\pm$ 0.05	1.79 $\pm$ 0.22	58†	NT	NT	NT
	6	<5	128	1.33 $\pm$ 0.68	2.91 $\pm$ 0.57	54†	NT	NT	NT
	7	<5	32	0.47 $\pm$ 0.06	0.73 $\pm$ 0.02	36†	13.2	1.7	7.8
	8	<5	128	0.31 $\pm$ 0.01	0.40 $\pm$ 0.01	22†	6.3	1.7	3.7

*Mean area of macrophage migration $\pm$ standard deviation.

†Significant inhibition of macrophage migration ($P < 0.05$).

NT, not tested.

Table 3 Effect of Ribavirin on transplanted adenovirus 12-induced tumours of CBA mice

Experiment no.	Ribavirin treatment	Incidence of tumours, days post-inoculation (mean tumour diameter in cm)					
		7	14	17	21	24	26
1	100 mg kg ⁻¹ daily from day -1 to day 21	0/8	0/8	1/8 (0.2)*	2/8 (0.2)	4/8 (0.4)	4/8 (0.7)
	Nil	0/8	2/8 (0.3)	4/8 (0.3)	5/8 (0.7)	6/8 (1.0)	7/8 (1.5)
2	100 mg kg ⁻¹ daily from day -1 to day 21	0/8	0/8	0/8	2/8 (0.1)	5/8 (0.3)	5/8 (0.5)
	Nil	0/8	2/8 (0.1)	4/8 (0.3)	5/8 (0.5)	8/8 (1.0)	8/8 (1.4)

*No. of mice with tumours/no. of mice inoculated (mean tumour diameter in cm)

the concentration and by the route of inoculation used, inhibited the serum antibody response to influenza virus vaccine, but did not inhibit the cellular immune response.

Previous studies have shown that several compounds, such as ethidium bromide, rifamycin and Tilorone inhibit the RNA-dependent DNA polymerase of oncornaviruses and have an antitumour activity⁷⁻¹⁰; indeed, if oncornaviruses are important in maintaining the cancerous properties of transformed cells, the two properties may be related. Thus, Tilorone has a chemotherapeutic action against a wide range of viruses, and is an interferon inducer¹¹; however, the antitumour activity of different analogues of this compound correlated more closely with the inhibitory activity on viral DNA polymerase, than with the ability to induce interferon¹². From these observations, it is possible that many antiviral compounds, such as Ribavirin, may have antitumour activity. To test this possibility, groups of CBA mice were inoculated subcutaneously with 100 mg kg⁻¹ d⁻¹ of Ribavirin. Two hours after the second drug inoculation, these animals, together with a group of untreated mice, were inoculated subcutaneously at a different site with 5 × 10⁵ transplanted adenovirus 12-induced CBA mouse tumour cells; the tumour cell inoculum used represented ten (50%) tumour doses¹³. Ribavirin was given to the treated mice for a further 20 d, and the incidence and size of tumours in the animals was recorded twice weekly for 4 weeks. Two identical experiments were carried out, and the results are shown in Table 3. In both experiments, the incidence of tumours and the size of palpable tumours was greater in control mice than in Ribavirin-treated mice; thus, Ribavirin inhibited the growth of transplanted adenovirus 12 tumours.

It is not certain from these studies that the antitumour activity of Ribavirin was due to the action of the drug on oncornaviruses present in the tumour cells; there are alternative mechanisms. The immune response of animals and man to either experimental or natural tumours has been shown to be cell mediated^{14,15}, and factors which promote the cellular immunity of the host may enhance tumour immunity¹⁶. In contrast, sera from tumour-bearing animals may contain blocking factors which interfere with the cell-mediated immune response, and the production of humoral antibody could enhance tumour growth¹⁷. By allowing a cell-mediated response to transplanted tumour cells and inhibiting the humoral antibody response, as shown in the present studies in guinea pigs, the antitumour activity of Ribavirin may be due to the effect of this compound on the immune response of the host, and an enhancement of the mechanism of tumour cell rejection. By which mechanism or combination of mechanisms Ribavirin exerts an antitumour activity is not known; however, it is possible that the effectiveness of the compound is a result of a direct action on the oncornavirus present in tumour cells. If this is so, other antiviral compounds may share this property, and this should be investigated.

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Relationship between the oncornavirus gene product gp70 and a major protein secretion of the mouse genital tract

SEVERAL investigators have postulated that endogenous oncornaviruses play a role in development and differentiation, and that neoplasia is an unfortunate consequence of an otherwise important symbiosis^{1,2}. Although attractive on theoretical grounds, these concepts remain unproven. Nevertheless, it is possible to study the relationship between viral gene expression and normal host functions. Evidence from several laboratories has suggested that expression of endogenous oncornavirus genes are under differentiation control in the mouse³⁻⁶. The clearest example of this is the case of gp70, the major envelope glycoprotein of the murine leukaemia viruses (MuLV)⁷⁻¹¹. This protein which is coded for by the viral genome¹² may be a component of the surfaces of cells which follow certain pathways of differentiation. Further, the thymocyte differentiation marker, G_{1X}, has been shown to be a type-specific antigenic determinant of some gp70 molecules^{4,5}.

Although, as studies of G_{1X} suggested, expression of gp70 is linked to cellular differentiation, we have shown already that its control is more relaxed than previously recognised. By immunofluorescence, gp70 was detected in various cell types, particularly lymphoid and epithelial

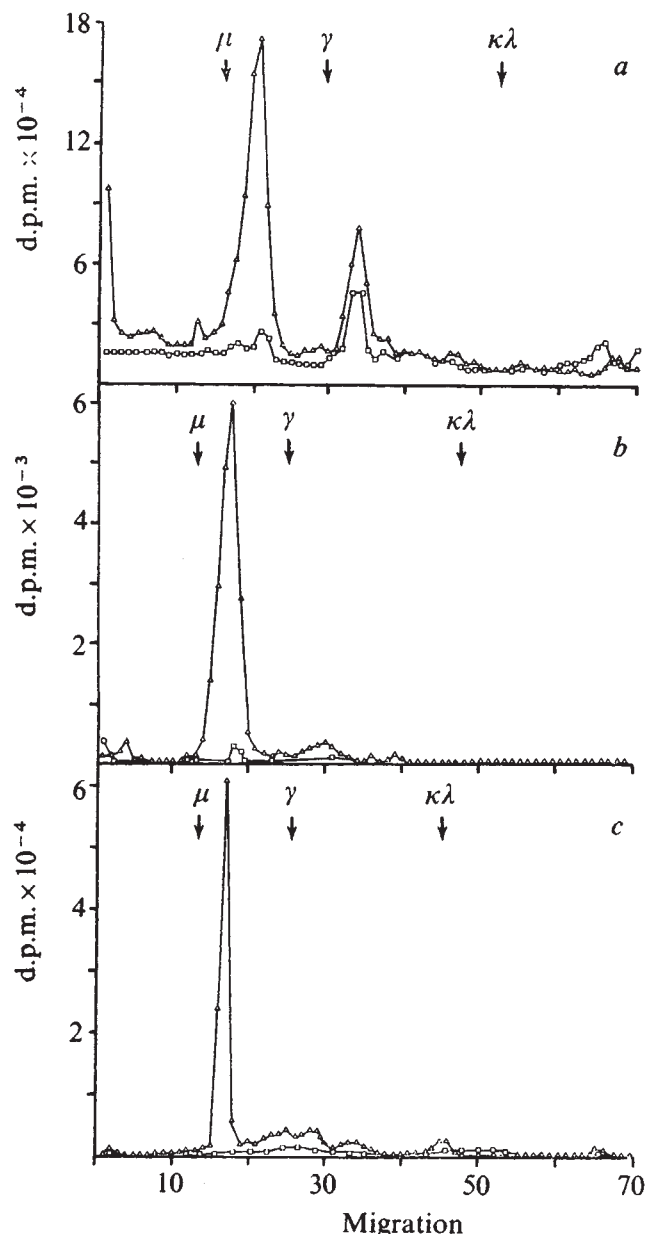


Fig. 1 Characterisation of MuLV gp70 in secretions of epididymis and ductus deferens. Secretions of the epididymis and ductus deferens of an NZB mouse and disrupted MuLV (Scripps) were radioiodinated by the chloramine T method¹⁴. MuLV-producing lymphoblastoid cells (SCRF 60 A) were surface radioiodinated using lactoperoxidase as described previously^{6,15}. After washing, cells were disrupted with 0.5% Nonidet P-40 and sonication, then centrifuged at 100,000g for 1 h. The resulting supernatant was used for immune precipitation. Labelled preparations were analysed by indirect immune precipitation as described in detail before^{6,8}. Either goat anti-gp70 (Scripps) or normal goat serum was used as the primary antibody and rabbit anti-goat IgG was the second antibody. Resulting immune precipitates were dissociated in 8 M urea, 1% SDS, 2% β -mercaptoethanol, and then analysed on 6 $\times$ 100 mm SDS polyacrylamide using the Laemmli system¹⁶. ¹²⁵I-radioiodinated μ , γ , and $\kappa\lambda$ marker proteins from human IgG were included in each gel. Gels were sliced and counted as described before^{6,8}. Results were corrected for crossover and counting efficiency and were plotted with a Hewlett-Packard system. Position of the marker protein is indicated by arrows. *a*, Radioiodinated SLV proteins reacted with goat anti-gp70 (Δ) or with normal goat serum ($\square$). This figure shows that the goat anti-gp70 reacts primarily with MuLV gp70. The gp45 in this gel is a breakdown product of gp70, a subject which is now being studied. *b*, Radioiodinated SCRF 60 A cell surface proteins reacted with goat anti-gp70 (Δ) or with normal goat serum ($\square$). Again, the specificity of the goat anti-gp70 for MuLV gp70 is shown. *c*, Radioiodinated secretions from the genital tract of a NZB mouse reacted with goat anti-gp70 (Δ) or with normal goat serum ($\square$). This figure demonstrates that gp70 is a component of the secretions of the male genital tract. The approximate molecular weight of the gp70 from each source is 67,000.

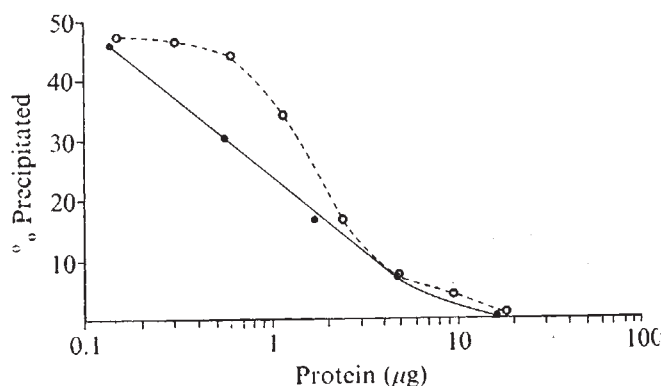
cells¹³. We now report that a protein related immunologically to gp70 is present in the secretions of the epididymis and ductus deferens of the mouse. This protein has a molecular weight of 67,000, comprises up to 10% of these secretions, and is associated with the surface of sperm. Apart from its biological importance, identification of a major site of expression of an oncornavirus-related protein allows us to complement serological analysis with a structural study in which a virion structural protein is compared with a differentiation antigen of the mouse.

The molecular properties of the gp70 molecules of MuLV and of the secretions of the epididymis and ductus deferens were compared by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis of indirect immune precipitates and by analysis of peptide maps of proteins eluted from gels. The data in Fig. 1 show that the anti-gp70 antibody reacts specifically with a single component from each of three sources: (1) purified MuLV (Scripps), (2) surface radioiodinated MuLV producing lymphoblastoid cells (SCRF 60 A) or (3) secretions from the epididymis and ductus deferens. The molecular weight of the protein was approximately 67,000 in all three cases. A comparison of the tryptic peptides of AKR virus gp70 and genital tract protein from AKR mice showed significant differences (B.C.D.V. *et al.*, in preparation). We do not yet know if the observed differences are due to glycosylation or change in the amino acid composition.

Antigenic cross reactivity between MuLV gp70 and the gp70 of the genital tract was confirmed by double diffusion studies. Antibodies against purified MuLV gp70 gave a reaction of partial identity between MuLV gp70 and a molecule in the secretions of epididymis and ductus deferens. MuLV gp70 has antigenic determinants not present on the genital tract protein, resulting in a 'spur' in the Ouchterlony plate. After absorption of anti-gp70 antibody with a crude preparation of secretions from the epididymis and ductus deferens, the reaction between the absorbed antiserum and the genital tract protein was abolished whereas the reaction between the absorbed antiserum and the viral gp70 was not. Conversely, we showed that absorption of the anti-gp70 with virus abolished reaction to both viral and genital tract protein.

The amount of gp70 in preparations of the secretions from the genital tract was determined by competition radioimmunoassay using the interspecies assay. As discussed by Strand and August⁹, such competition curves are the result of three parameters of the reaction. First, the concentration of the competing proteins; second, the relative affinity of the antibodies for the proteins, and, third, the composition of the antigenic determinants of the proteins and of the

Fig. 2 Competition radioimmunoassay for MuLV gp70. Reaction conditions were similar to those of Strand and August⁹ in which the interspecies determinants of gp70 are measured; goat anti-FuLV (1:700) and ¹²⁵I-gp70 (Friend) (1 ng). Competing proteins were from MuLV (Scripps) ($\circ$) and from secretions of epididymis and ductus deferens of an NZB mouse ($\bullet$). Similar curves for the genital tract gp70 were obtained for NZB (NZB $\times$ NZW)F₁, NZW and 129 G₁X⁺.



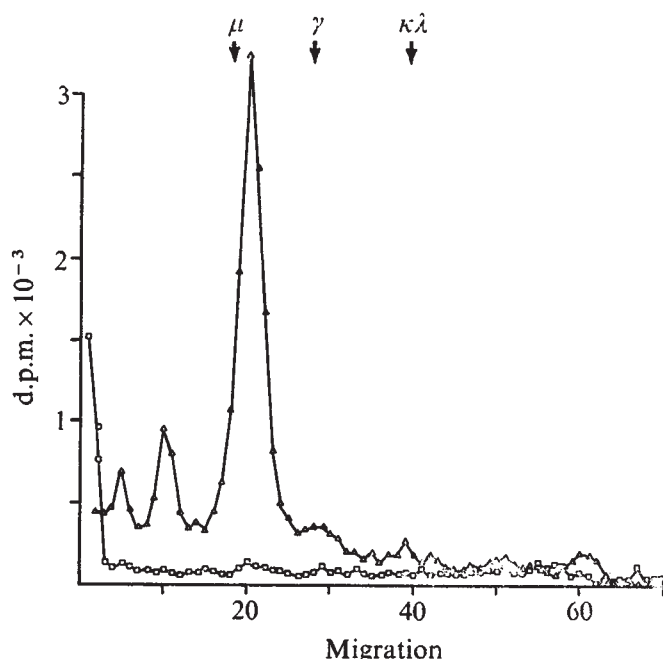


Fig. 3 Association of MuLV gp70 with sperm. NZB sperm were purified from epididymis and ductus deferens by differential centrifugation. Sperm were surface radioiodinated with lactoperoxidase, then extracted and analysed. SDS polyacrylamide gel electrophoresis of radioiodinated sperm proteins reacted with goat anti-gp70 (Scripps) (Δ) or with normal goat serum ($\square$). Position of ^{125}I marker proteins is indicated by arrows. The approximate molecular weight of the sperm gp70 is 67,000.

antibodies. A set of representative binding curves is shown in Fig. 2. The relative amount of gp70 in the secretions of the genital tract varied considerably from preparation to preparation, ranging from 2 to 10% of the protein. This variation may reflect different amounts of gp70 in the secretions from mouse to mouse, and from strain to strain, but was also undoubtedly influenced by the extent to which the secretions were recovered and/or contaminated by other proteins, especially serum proteins.

The relative slope of the inhibition curves for MuLV gp70 and that of the genital tract proteins are different, indicating differences in the affinities of the interspecies antigenic determinants for the antibodies. At high concentrations the genital tract proteins compete completely for antibody with the labelled viral gp70. Thus, most of the viral interspecies determinants are present in the genital tract protein. These studies show that the MuLV gp70 and the molecules from the genital tract are immunologically similar, but not identical.

Proteins of epididymal secretions are known to associate with the surface of sperm where they may play a role in capacitation. To determine whether gp70 associated with the sperm surface, epididymal sperm were purified and then surface radioiodinated. Figure 3 shows that in this strain, a gp70 molecule is associated with the sperm surface. It is interesting that studies (to be reported elsewhere) have shown that the gp70 of the epididymal secretions and that associated with the sperm surface may be the product of different genes.

The basic question relating to the proteins studied here is whether the host and viral proteins are transcripts from a single gene and are modified after synthesis as by glycosylation, or whether they are products of different genes. The differences in peptide maps strongly suggest that they are different gene products. A possibility not excluded by our studies is that MuLV gp70 and the genital tract protein are unrelated gene products modified so as to cross react immunologically. But a trivial cross reactivity seems un-

likely for several reasons. First, three different antisera prepared against purified gp70 from three different FMR viruses all reacted with the genital tract protein. Second, we know from previous studies that at least one protein of the male genital tract shows a mode of inheritance identical to that of G_{IX} and is lost in congenic G_{IX}^- mice¹³. Third, the interspecies determinants of viral gp70 are completely represented in the genital tract protein. Finally, the cross reactivity between viral and genital tract gp70 is not simply due to common carbohydrate groups. Unpublished results from our group and Bolognesi's group (personal communication) show that proteolysis destroys the antigenicity of gp70 whereas removal of the carbohydrate does not.

The data presented here indicate that several distinct genes for gp70 can be found in the mouse genome, some of which code for proteins made by certain differentiated cells, and others which code for proteins which are incorporated into virus.

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Isolation of nucleic acid-binding protein: stimulation of reverse transcriptase-catalysed DNA synthesis

RNA tumour viruses transfer their genetic information from a single-stranded RNA genome found in their virions to a double-stranded DNA covalently integrated into chromosomes of the infected host^{1,2}. A series of nucleic acid intermediates must therefore exist between the single-stranded RNA and the integrated viral DNA genome. The discovery of RNA-directed DNA polymerase (reverse transcriptase) associated with RNA tumour viruses fulfilled a critical requirement for synthesis of viral DNA^{3,4}. The initial product in this information transfer is a RNA DNA

hybrid which is converted into free, unintegrated DNA-DNA^{3,5}. The double-stranded DNA (provirus) is then integrated into chromosomes of the infected host. We report here the isolation of a nucleic acid-binding (unwinding) protein from chick fibroblasts transformed by Rous sarcoma virus (RSV) and its stimulatory effect on DNA synthesis catalysed by reverse transcriptase. A protein capable of unwinding RNA-DNA hybrid and DNA-DNA duplex would not only conserve the input viral RNA strand for further reverse transcription, but also facilitate replication of the double-stranded viral DNA into many copies of provirus before integration.

Binding protein was isolated by affinity chromatography from chick embryo fibroblasts chronically transformed by Schmidt-Ruppin RSV, subgroup D after 2 weeks of virus infection. Cellulose containing covalently linked single-stranded calf thymus DNA was purchased from P.L. Biochemicals. All radioactive polynucleotides were purchased from Miles Laboratories. In a typical run about 2 wet g transformed cells were washed, sonicated and centrifuged to obtain debris-free supernatant. The supernatant was treated with polyethylene glycol and centrifuged to remove nucleic acid. After dialysis the supernatant was

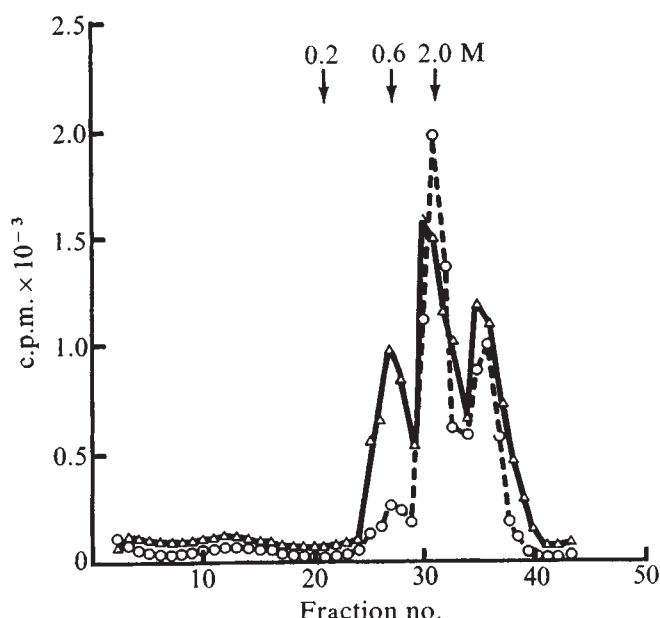


Fig. 1 Isolation of DNA-binding protein using DNA-cellulose column. Frozen chicken embryonic fibroblasts (2 g), completely transformed by RSV, subgroup D, were suspended and washed twice with phosphate-buffered saline. Cells suspended in 4 ml sonicating buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM 2-mercaptoethanol, 1.7 M NaCl) and disintegrated for 45 s at 4 A in a Branson Sonifier. Cell debris removed by centrifugation at 10,000g for 10 min. Polyethylene glycol (MW 6,000–7,500, 30% in 1.7 M NaCl) was stirred into supernatant to a concentration of 10%. After stirring for 30 min, suspension centrifuged at 27,000g to remove nucleic acid precipitate. Resulting clear supernatant fraction dialysed against 2 l Tris buffer (20 mM, Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM 2-mercaptoethanol, 50 mM NaCl, 10% glycerol) for 20 h. Precipitate formed during dialysis removed by centrifugation (27,000g for 20 min) and supernatant passed through (4 ml h⁻¹) a column (1 × 1 cm) of single-stranded DNA-cellulose (P.L. Biochemicals) that had been washed with the Tris buffer. Column washed with 10 ml Tris buffer and eluted with 2.5 ml each of 0.2 M, 0.6 M, 2.0 M NaCl in Tris buffer. Fractions (0.5 ml) were collected. ³H-poly d(AT)-poly d(AT) (2 nmol, 25 mCi mmol⁻¹ P) or ³H-poly(rA)-poly(dT) (2.8 nmol, 18 mCi mmol⁻¹ P) incubated with fractions (10 μl) in binding buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2 mM mercaptoethanol, 50 mM NaCl and 5% glycerol) in total volume 0.1 ml at 35 °C for 10 min. Solution chilled and diluted with 2 ml binding buffer and filtered through Millipore filter. Filter washed 3 times with 3 ml binding buffer, dried and radioactivity determined in toluene-based scintillation fluid. Δ, Binding of ³H-poly d(AT)-poly d(AT); ○, binding of ³H-poly(rA)-poly(dT).

Table 1 Effect of binding protein (BP) on DNA synthesis by reverse transcriptase

Template	pmol ³ H-dTMP incorporated per h*		Ratio + BP – BP
	– BP	+ BP	
Native calf thymus DNA	0.86	1.50	1.7
Nicked calf thymus DNA†	0.88	2.62	3.0
Heated calf thymus DNA‡	0.80	11.0	13.7

*Reaction conditions as in Fig. 2 except 0.9 U reverse transcriptase were used. Binding protein, when present, was 0.5 μg per incubation. DNA concentrations were 50 μg ml⁻¹.

†Calf thymus DNA nicked by treatment with DNase I (ref. 11).

‡Calf thymus DNA was heat-denatured at 100 °C for 15 min and chilled immediately¹¹.

passed through a DNA-cellulose column (1 × 1 cm) pre-equilibrated with Tris buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM 2-mercaptoethanol, 50 mM NaCl and 10% glycerol). The column was washed with the Tris buffer and then eluted stepwise with 0.2, 0.6 and 2.0 M NaCl in the buffer. Resulting fractions were assayed for nucleic acid binding activity as described previously⁷ using either ³H-poly d(AT)-poly d(AT) or ³H-poly(rA)-poly(dT) as substrate. Fig. 1 shows the results obtained from the DNA-cellulose column. There were three peaks of binding activity when the radioactive templates were used. All the fractions containing binding activity were combined and dialysed against the binding protein buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM 2-mercaptoethanol, 20 mM NaCl and 30% glycerol).

Table 2 Effect of binding protein (BP) on reverse transcription of RSV 70S RNA

Template	Binding protein	pmol ³ H-dTMP incorporated*	+ BP % – BP
70S†	0	0.65	—
70S	0.8 μg	1.08	1.6
70S	2.0 μg	1.54	2.4
—	2.0 μg	0	—

*Reaction conditions as in Fig. 2, except 0.5 U purified reverse transcriptase from RSV B77 used.

†RSV 70S RNA (4.5 μg ml⁻¹) isolated from RSV B77 by sucrose density centrifugation²⁴; 70S peak showed a single band of high molecular weight material in gel electrophoresis²⁵.

The effect of the isolated binding protein on polymerase activity was studied in a reaction in which reverse transcriptase⁸ catalysed DNA synthesis using heat-denatured calf thymus DNA as template. DNA synthesis as represented by TCA-insoluble counts⁹ was linearly proportional to the amount of binding protein¹⁰ present in the reaction with a maximum stimulation of about tenfold increase with 1 μg of the protein (Fig. 2). Heating of the binding protein at 100 °C for 5 min completely abolished the stimulatory effect and the binding protein itself contained no polymerase activity (Fig. 2).

It is clear from Figs 1 and 2 that the isolated protein binds to DNA and stimulates reverse transcriptase in DNA synthesis. We explored the possibility whether the binding protein enhances DNA synthesis by promoting denaturation (unwinding) of double-stranded DNA. When native calf thymus DNA was used as template the binding protein stimulated DNA synthesis by reverse transcriptase about twofold, which is not inconsistent with the unwinding of the template (Table 1). When the template was native DNA nicked slightly with DNase¹¹, the protein stimulated DNA synthesis even greater (threefold), suggesting easier unwinding of the double-stranded DNA after nicking. When heat-denatured DNA was used as template, denatured DNA in the absence of the protein was a poor template for

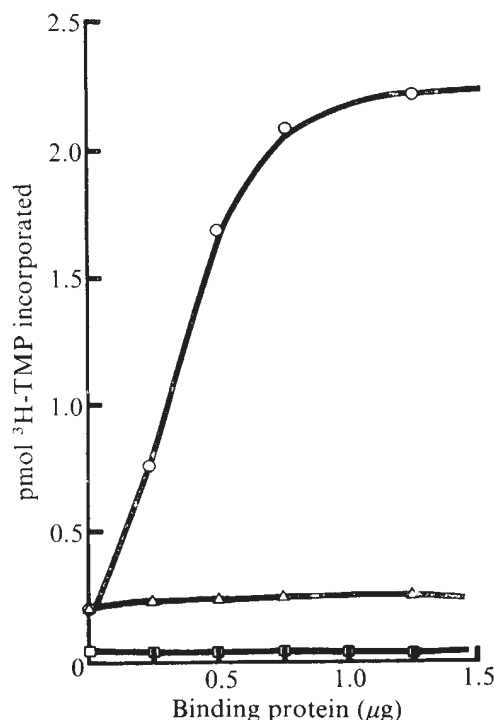


Fig. 2 Stimulation of reverse transcriptase DNA synthesis by binding protein. Reaction mixture in total volume of 120 μ l contained purified reverse transcriptase⁸ (phosphocellulose fractions, eluted with 0.22 M phosphate buffer), 0.3 U; dATP, dGTP, dCTP, 0.1 mM each; MgCl₂ 5 mM; dithiothreitol, 3 mM; NaCl, 50 mM; heat-denatured calf thymus DNA, 6 μ g (Sigma, type 1, boiled at 100 °C for 15 min and chilled); ³H-dTTP, 8 μ Ci (20 μ Ci nmol⁻¹); and specified amount of binding protein as determined by Lowry *et al.*¹⁰. Incubation carried out at 35 °C for 60 min and TCA-insoluble radioactivity determined by filter paper method. One unit of reverse transcriptase activity was defined as ability to incorporate 1 pmol ³H-dTTP into TCA-insoluble precipitate per h in experimental conditions described above. ○, Reverse transcriptase activity in presence of binding protein; △, in presence of heated binding protein; □, polymerase activity of binding protein alone.

reverse transcriptase, which is in agreement with the observation that reverse transcriptase did not prefer single-stranded DNA¹². Binding protein, however, stimulated greatly (14-fold) DNA synthesis when heat-denatured DNA template was used. We suggest that the protein binds strongly and cooperatively to single-stranded DNA and thus promotes unwinding of the small region of undenatured, double-stranded segment to which reverse transcriptase is attached. When bound to single-stranded DNA, it prevents intrastrand annealing and maintains the DNA in an extended conformation, thereby making the template more transcribable.

That the binding protein can unwind double-stranded DNA was studied in a separate experiment. When an excess of the binding protein (78 μ g) was added at 24 °C to 8 μ g double-stranded DNA, poly d(AT):poly d(AT), A_{260} of the DNA increased as a function of time until a plateau 35% greater than the original absorbance was reached (Fig. 3). Similar results were obtained using poly(rA):poly(dT), poly(A):poly(U) and 70S RSV RNA as substrates; the hyperchromic effects were about 29%, 19% and 20%, respectively, in the conditions used (not shown here). This hyperchromic shift is characteristic of the helix-coil transition which has been observed with the binding protein from bacteria¹³⁻¹⁵.

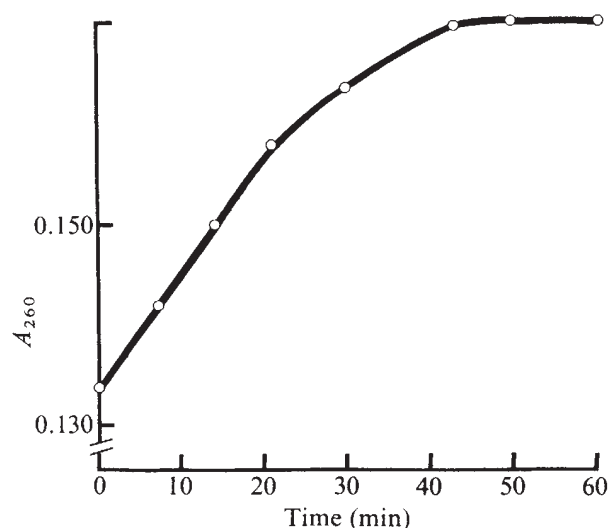
The binding protein reported here was capable of binding to all forms of nucleic acids: double-stranded DNA, ³H-poly d(AT):poly d(AT), single-stranded DNA (affinity

column), double-stranded RNA (³H-poly(A):poly(U)), single-stranded RNA (³H-poly(A)) and DNA-RNA hybrid (³H-poly(rA):poly(dT)). The binding affinity in preliminary studies indicated about the same preference by the protein for double-stranded DNA or RNA-DNA hybrid. The affinity to single-stranded RNA (³H-poly(U)), however, was greater than double-stranded RNA (³H-poly(A):poly(U) or ³H-poly(I):poly(C)).

The protein isolated by the DNA affinity column contained some DNase activity. Details of purification and properties will be described elsewhere. As in the case of the reported specificity of binding proteins to polymerases from the same source^{13,15-18}, the binding protein stimulated greatly reverse transcriptase but little, if any, polymerase from *M. lysodeikticus* (data not shown). Although the binding protein stimulated sevenfold DNA synthesis by bacterial polymerase using native DNA as template, it stimulated little, if any, DNA synthesis after the template was heat denatured, suggesting that the stimulation was attributable to unwinding of the template by the protein instead of interaction between the binding protein and the bacterial polymerase. In contrast to this, the binding protein stimulated 14-fold DNA synthesis by reverse transcriptase on the heat-denatured template, suggesting that the binding protein, in addition to unwinding DNA, may also bind to reverse transcriptase during synthesis in such a way that the enzyme does not dissociate from the template and thus prolongs synthesis. We are studying the possibility of such an interaction between reverse transcriptase and the binding protein analogous to that of polymerase II and the binding protein in *Escherichia coli*^{15,16}.

We have yet to determine the exact relationship of the binding protein reported here to viral RNA replication. We have also isolated a binding protein from chick embryo fibroblasts similar to that from RSV-transformed cells. Therefore, it seems that the binding protein is not coded by the virus. Intramolecular hydrogen bonding of 70S RSV genome is well known¹⁹. In the presence of the binding protein in a preliminary study, we have observed in electron microscopy an extended form of RSV RNA instead of the usual, collapsed structure, similar to the observation of

Fig. 3 Hyperchromicity of poly d(AT):poly d(AT) in presence of DNA-binding (unwinding) protein. Reaction mixture contained alternating copolymer (8.0 μ g) and binding protein (78 μ g) in 1.2 ml buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM mercaptoethanol, 20 mM NaCl, 30% glycerol). A_{260} determined with a recording Gilford spectrophotometer at 24 °C immediately after mixing of copolymer and binding (unwinding) protein at 24 °C. Cuvettes containing copolymer or binding protein alone in buffer were also scanned to calculate increase in absorbance.



RSV RNA in the presence of gene 32 protein¹⁹. Probably due to the collapsed RNA structure, a disturbing feature of reverse transcriptase reaction *in vitro* has been the relatively small size (5–7S) of the product and the faster initial rate of DNA synthesis^{20–23}. In the presence of the binding protein, however, the rate of synthesis and the size of the DNA product would be expected to increase. In our preliminary studies using RSV 70S as template, increasing amounts of the binding protein stimulated linearly the rate of DNA synthesis in a homologous reverse transcriptase reaction (Table 2). A detailed study of the influence of the binding protein on the size of DNA products and the kinetics of synthesis is under way.

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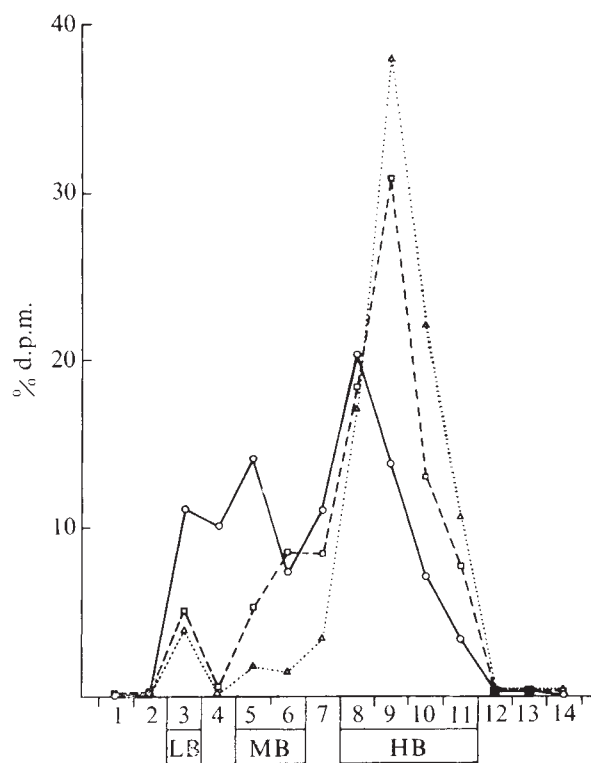


Fig. 1 Distribution of radioactivity in DNA of nuclear sub-fractions after 5, 30 or 120 min of labelling *in vivo*. Female Osborne-Mendel rats weighing 120 g were injected intra-peritoneally with tritiated thymidine 23 h after partial hepatectomy. Nuclei were isolated from the livers by the method of Lynch *et al.*¹⁰. Nuclear pellets were suspended in 5 mM Tris-HCl, pH 7.5 containing 5 mM Na₃EDTA and then sonicated in precisely defined conditions⁵. Cs₂SO₄ was added to this suspension to achieve a 1.3 g cm⁻³ solution, and nuclear constituents were allowed to concentrate at their characteristic intrinsic buoyant densities in the gradients generated during a 66-h centrifugation at 35,000 r.p.m. in an SW40 rotor at 25 °C. The gradients were fractionated into 1-ml portions, and processed as follows: precipitating and washing with cold dilute TCA and PCA to remove Cs₂SO₄ and unincorporated radioactivity; treatment with 0.3 M NaOH at 37 °C for 1 h followed by further washing with acid, and hydrolysis of DNA with 1.2 M PCA at 60 °C for 1 h. Radioactivity and DNA content were quantified in this hydrolysate. Nearly all radioactivity recovered after our standard processing was present in DNA and in thymidine residues when this DNA was subjected to enzymatic hydrolysis and chromatography (unpublished observation). The percentage of the total gradient d.p.m. present in each fraction was plotted to demonstrate the distribution of radioactivity after 5 (○), 30 (□), and 120 (△) min of labelling. The total d.p.m. incorporated were 129, 191 at 5 min, 357,586 at 30 min and 1,749,712 at 120 min. The portions of the gradients corresponding to the light band (LB), middle band (MB) and heavy band (HB) are marked at the bottom of the figure.

Eukaryotic DNA replication complex

We postulate that eukaryotic DNA replication occurs at discrete intranuclear macrostructures, replication complexes, which might be separable from the bulk of nuclear material. A complex composed of DNA, protein and other nuclear constituents could have a different intrinsic density from nucleic acids or protein alone, and this might be the basis for its isolation. Replication complexes would characteristically contain nascent DNA^{1–4} and DNA polymerase. If they are functionally intact, they should also have the intrinsic capacity to synthesise DNA. We have used a previously reported method for the fractionation of sonically disrupted nuclei on gradients of Cs₂SO₄ (ref. 5). The results indicate that replication complexes may be partially purified by this method, and that ATP-dependent DNA synthesis is a characteristic of the complexes.

Isolated nuclei from regenerating rat liver were sonicated carefully and the sonicate was banded in equilibrium density gradients of Cs₂SO₄ (ref. 5). Three principal bands were observed in the gradients: light band (LB), middle band (MB) and heavy band (HB) (average densities: 1.21, 1.26, and 1.32 g cm⁻³, respectively). These bands possess different relative concentrations of DNA, RNA and protein⁵. To demonstrate nascent DNA, rats were labelled with tritiated thymidine for 5, 30 or 120 min *in vivo* 23 h after partial

hepatectomy (DNA synthesis peak⁶). The percentage distribution of radioactivity incorporated into DNA of gradient fractions changes with duration of labelling (Fig. 1) and this suggests a precursor-product relationship. LB (which contains 4.9% of the total DNA) has 11.1% of the radioactivity incorporated into DNA after 5 min and this decreases to 3.9% after 120 min; MB (which contains 7.9% of the total DNA) has 21.6% after 5 min and only 3.4% after 120 min; in contrast, HB (which contains 86% of the total DNA) has 44.9% at 5 min and this increases to 88.0% at 120 min. Much of the incorporated radioactivity is found in a small fraction of the total DNA after a short labelling period. With time there is a reduction in the proportion of radioactivity associated with the MB and LB DNA, and an increase in the proportion of radioactivity associated with the bulk DNA.

Previous reports^{1–4} indicate that nascent eukaryotic DNA

is partially "destabilised" and seems to have partial single-stranded character because of its ease of denaturation. To evaluate this property, DNA was purified from the Cs_2SO_4 gradient bands by rebanding in CsCl gradients. The purified DNA was then analysed by hydroxyapatite chromatography⁷ and gel electrophoresis⁸. For both LB and MB, a sizeable proportion (15–20%) of the DNA labelled for 5 min *in vivo* demonstrated limited mobility in acrylamide–Agarose gels, consistent with the limited mobility demonstrated for replication forks from *Escherichia coli*⁹. In addition, a larger proportion of LB and MB DNA than HB DNA was more readily eluted from hydroxyapatite than native double-stranded DNA, which suggested a greater partial single-stranded character to LB and MB DNA.

The intranuclear localisation of the labelled product of DNA replication and repair *in vitro* in isolated rat liver nuclei was evaluated in Cs_2SO_4 gradients. DNA replication (semi-conservative) in isolated S-phase liver nuclei is ATP dependent^{10–11} whereas DNA repair (non-semi-conservative) is ATP independent and best observed in non-S-phase nuclei^{11–12}. Analysis of Cs_2SO_4 gradients of sonicated nuclei after DNA replication *in vitro* showed the labelled DNA product of the ATP-dependent process to be largely confined

to the MB and LB (Fig. 2). In contrast, the labelled DNA product of non-semi-conservative DNA synthesis in isolated nuclei roughly paralleled the total distribution of DNA in the gradient and thus did not show specificity of localisation (Fig. 2). In previous experiments³ we have shown the presence of DNA polymerase activity (primed with exogenous activated DNA) in gradient fractions of unlabelled liver nuclei and have shown the greatest quantities to be in MB and LB. In other experiments gradient fractions from unlabelled regenerating rat liver nuclei were evaluated for endogenously-primed, ATP-dependent DNA synthesis in assay conditions used for comparable nuclei. In the approximately 50% of these studies in which ATP-dependent DNA synthesis was detected in the gradient fractions, the MB was consistently shown to have the highest levels of this activity (unpublished observation).

The presence of ATP-dependent DNA synthesis and partially single-stranded, rapidly labelled DNA containing replication forks in the same fractions from Cs_2SO_4 gradients indicates that this procedure partially purified eukaryotic DNA replication complexes from S-phase rat liver nuclei. This result extends the observation of ATP-dependent DNA replication in isolated mammalian cell nuclei^{10,11,13} and complements the observation of ATP-dependent DNA synthesis in MB fractions isolated from sea urchin embryo nuclei¹⁴. Since nuclear membrane is largely confined to the LB (unpublished observation), the result reported here differs from studies involving the MB technique^{14–16} because a sizeable proportion of nascent DNA is in the MB as well as in association with the nuclear membrane (in the LB). The property of ATP-dependent DNA synthesis seems to provide a characteristic *in vitro* assay with which the replication complex can be purified. In future experiments we will attempt to determine whether other enzymes, such as deoxyribonuclease, DNA ligase, DNA-dependent RNA polymerase, RNase H, as well as DNA-unwinding protein are intrinsic constituents of the DNA replication complex. Identification of constituents of the replication complex and preparation of appropriately labelled antibodies to them may allow unambiguous localisation of DNA replication sites in the nucleus by direct visualisation.

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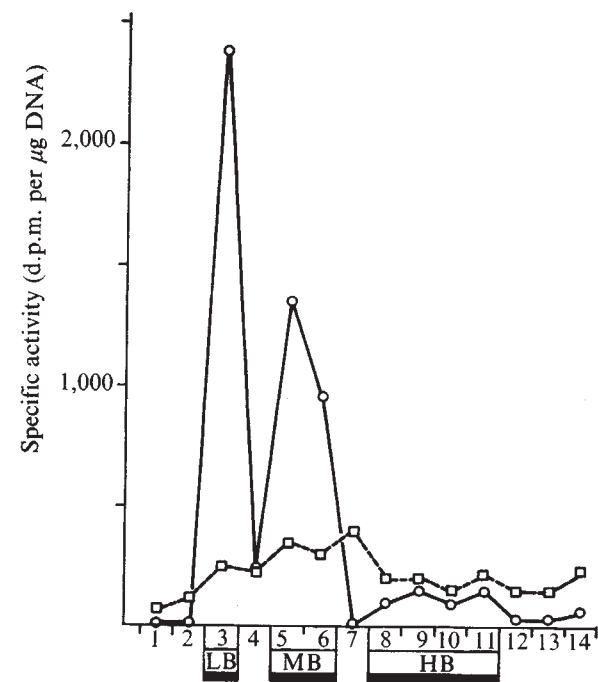


Fig. 2 Distribution in Cs_2SO_4 gradients of DNA product of semi-conservative and non-semi-conservative DNA synthesis *in vitro*. Nuclei were isolated¹⁰ from normal rats and from partially hepatectomised rats 23 h after operation. Nuclei from normal liver were treated with DNase I to stimulate non-semi-conservative, ATP-independent DNA synthesis¹¹. Regenerating liver nuclei were incubated with and without ATP in conditions previously used to demonstrate ATP-dependent DNA replication¹¹. For non-semi-conservative DNA synthesis DNase-treated normal liver nuclei were assayed in the same conditions, but with ATP omitted. After 5 min incubation at 37 °C, nuclei were cooled in an ice bath, pelleted by centrifugation at 600g and then resuspended in 5 mM Tris-HCl, pH 7.5 containing 5 mM Na_3EDTA . Nuclei were sonicated and the sonicates were banded in Cs_2SO_4 equilibrium density gradients⁹. The gradients were then fractionated into 1-ml portions and the fractions were processed as described in the legend to Fig. 1. DNA content and radioactivity were determined for each fraction. ATP-dependent DNA replication in regenerating rat liver nuclei was determined as the activity of nuclei incubated with ATP minus the activity without ATP¹¹. The d.p.m. in each fraction were normalised to the DNA content of the fraction and the specific activities were plotted for ATP-dependent DNA replication (○) and DNase I-activated DNA synthesis (□). The total d.p.m. was 76,800 for ATP-dependent DNA replication and 44,800 for ATP-independent DNase-activated DNA synthesis.

Evidence for defined lengths of DNA replication units in 'satellite' DNA from *D. ordii*

EUKARYOTIC DNA can be isolated as long fibres on which replication has occurred at several locations¹⁻⁹. These replicating units are arranged in tandem and spaced at intervals which vary in length from 1–100 μm ^{2-5, 7-9}. Although it can be determined that at any time replication has been initiated from a particular position on a fibre, it is not known whether that position remains fixed from one round of replication to the next, nor whether replication terminates at fixed locations on the DNA fibre. Attempts to resolve these questions have used experiments in which replicated units are visualised and their length and spatial distribution reconciled with models of fixed^{4,5} or varying^{3,8} sites of initiation and termination. Different studies have reached the different conclusions that these sites are variable^{3,8} or fixed^{4,5} (particularly with respect to termination of replication). All of these studies have been complicated by the observation that the length of replicated regions seems to vary in a manner consistent with a broad but continuous distribution

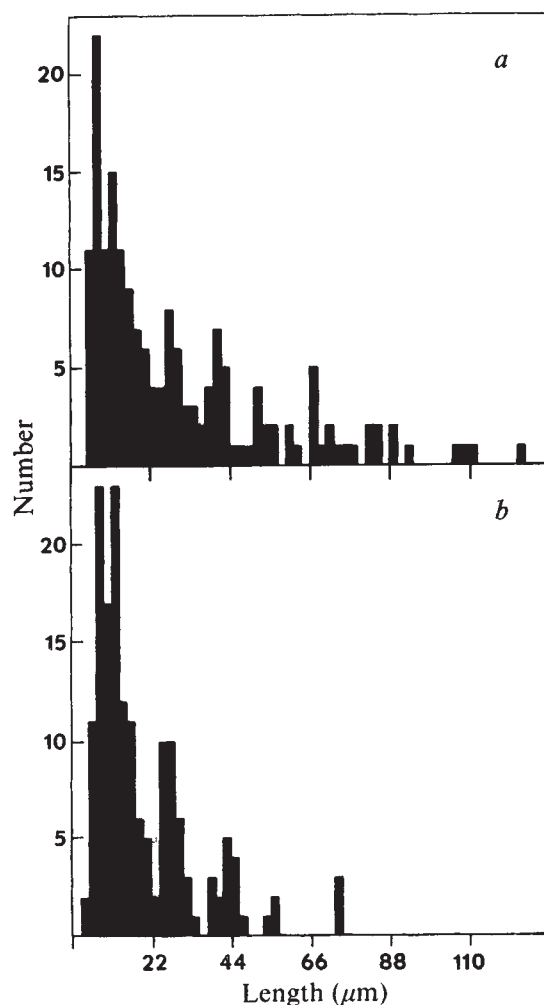


Fig. 1 Frequency of *a*, non-replicated regions (GAPS) and *b*, replicated (radioactive) regions assorted with respect to length. Cells of *D. ordii* grown as monolayers were inhibited with FUDR (1 $\mu\text{g ml}^{-1}$) for 12 h. Inhibition was reversed by addition of ³H-thymidine (50 Ci mmol⁻¹; 1 $\mu\text{g ml}^{-1}$) and labelling continued for 8 h. DNA was isolated as described previously and autoradiographs prepared. The density profile of this material has been published (ref. 5, Fig. 2). These data were obtained from the fractions designated as S* in that publication. All of the radioactive units were taken from tandem arrays and only internal units were scored.

for the length of the replicating unit. Such a distribution could reflect variation in the position of fixed termination sites or the fact that termination is not at a fixed position on the DNA fibre.

We have isolated and visualised a set of DNA fibres in which the length and spacing of tandem-replicated units are distributed as multiples of a single fundamental unit. These measurements were made on 'satellite' DNA of *D. ordii*^{10,11} which had been isolated by CsCl centrifugation. The material to be described was prepared in a series of experiments reported previously⁵.

We have already presented evidence that satellite DNA with a buoyant density heavier than that of main band DNA¹⁰ and characteristically replicated late in S phase¹¹ (6 to 8 h after release of fluorodeoxyuridine (FUDR) block) can be visualised as multiple short segments of replicated DNA arranged in tandem and interspersed with non-replicated material⁵. In that study we prepared DNA from cells which had been continuously labelled with ³H thymidine for 8 h after release from FUDR inhibition of DNA synthesis. This DNA was fractionated by CsCl centrifugation and autoradiographs prepared from the different fractions. An outstanding characteristic of this material was the pattern of very long DNA fibres with small replicated and non-replicated regions occurring as tandem arrays on one fibre. We have quantitatively re-examined the material from the densest of these satellite fractions and obtained the distribution of non-radioactive and radioactive regions shown in Fig. 1*a* and *b*. A detailed description of the isolation and preparation of this DNA has been published⁵ and a summary of this experiment is presented in the legend to Fig. 1.

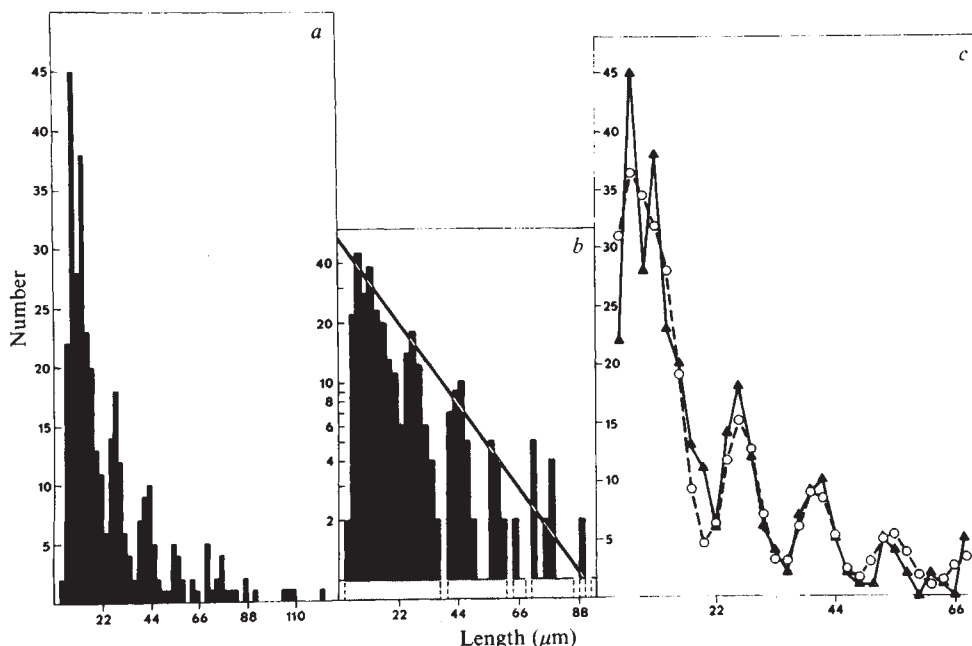
Both non-radioactive (Fig. 1*a*) and radioactive (*b*) regions are grouped into distinct size classes which seem to be the same. That this is the case is apparent when the data for both replicated and non-replicated material are combined (Fig. 2*a*). In this larger mixed population the discrete length classifications are even more apparent.

It is interesting that the frequency of any particular size class seems to decrease as an exponential function of its size (Fig. 2*b*). This relationship is apparent for all of the more heavily populated classes. The distribution in Fig. 2*a* was compared with the null hypothesis that pieces are distributed as a continuous function of their length according to the equation: number of any length, $L = Ae^{-\lambda L}$. The probability of obtaining the result in Fig. 2*a* assuming the null hypothesis to be correct is $P < 0.001$ ($\chi^2 = 93$, 27 d. f.). These data are based on a sample of 350 units distributed in tandem arrays on 31 fibres isolated after lysis of a population of 10^8 cells and fractionation of the DNA on a CsCl density gradient. During this process, the sample was thoroughly mixed and it is extremely unlikely that any two fibres were isolated from the same cell. A fraction of the same relative density isolated from cells labelled for 10 h instead of 8 h, did not yield tandem arrays, but only long stretches of continuously radioactive material^{5,11}. This suggests that the tandem arrays found at 8 h are due to replicated (radioactive) and non-replicated (non-radioactive) regions and that the non-radioactive regions are replicated between 8 and 10 h.

The data in Figs 1*a* and *b* and 2*a* are readily explained by assuming the existence of tandemly arranged replication units of defined length only some of which have been replicated after 8 h incubation in label.

The distribution of sizes is interesting. The size classes approximate one-, two-, four-, six-, eight- and tenfold multiples of a unit the length of which is about 7 μm . Figure 2*c* compares the distribution of size classes in Fig. 2*a* with the distribution generated by a computer assuming that size classes are normally distributed ($\sigma = 3.27 \mu\text{m}$) around a mean length which is one-, two-, four-, six-, or eightfold multiple of 6.93 μm assuming a declining amplitude (peak height) equal to $50e^{-0.037L}$, where L is the piece size in μm . Distributions which fit the data better than that in Fig. 2*c* can be generated if one assumes that s. d. of each size class is a function of the size, being least for the class of shortest pieces.

Fig. 2 Combined distributions of replicated and non-replicated regions assorted with respect to length: *a*, linear; *b*, logarithmic frequency scales; *c*, comparison of observed frequency distribution with that generated on assumption that pieces are distributed in classes of one-, two-, four-, six- or eightfold multiples of $6.93 \mu\text{m}$ (see text for details). $\blacktriangle$, Observed frequency; $\circ$, best fit distribution generated by computer.



The distribution in Fig. 2*a* and *c* cannot be explained by the simple association of units of the smallest size class because odd multiples are missing. Thus absence of lengths corresponding to three, five or seven times this basic unit requires that the population be composed of at least two categories of units: that corresponding to a single $7\text{-}\mu\text{m}$ unit and those corresponding to groupings of basic units each one of which is approximately twice that size. We have observed that 25% of the smallest units are distributed on two fibres, whereas the rest are distributed on some 17 fibres and are found in every instance as isolated units (that is, $7\text{-}\mu\text{m}$ units, either radioactive or non-radioactive, are separated from each other by larger units). No other clustering of any size class was apparent.

To our knowledge, distributions similar to that in Fig. 2 have not been observed in other material. It seems likely that this distribution reflects the regular spacing of replication units in satellite DNA and that this regularity may be generated by the repetition of particular sequences. On this assumption, it is possible, to construct models which can account for the evolution of the distribution seen in Fig. 2.

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Low temperature specific heat anomalies in melanins and tumour melanosomes

THE melanins are of great interest because of their widespread presence in biological systems. In humans, melanin is found in nearly all areas where energy transduction occurs, for example, skin, inner ear, retina, as well as in several other areas such as the midbrain. The loss of melanin in the substantia nigra (midbrain) has been correlated with Parkinson's disease, and melanin-drug interactions in the stria vascularis (inner ear) have been implicated in deafness (ototoxicity)¹⁻³. Study of the physical properties of the melanins has yielded considerable insight into possible functional roles in biological systems³⁻⁵, and has led to the design of possible new treatments for the human malignant disease, melanoma, based on interactions between ultrasound and melanin binding drugs^{6,7}. These observations have indicated that the melanins play an active, rather than passive, role in biological systems and intensive study of their properties should lead to better understanding of their functions *in vivo*. Such studies are also lending some insight into problems associated with the solid-state physics of amorphous materials^{2,5}.

Studies of the physical properties of melanins show them to be highly stable structures, with a large density of free spins (10^{19} g^{-1} (ref. 8)), and also amorphous⁹. These properties seem to be relatively insensitive to the method of preparation¹⁰. Experiments have shown that melanins exhibit rather exotic electrical characteristics similar to those found in some amorphous structures.

Low temperature specific heat measurements on amorphous solids have led to the observation that in many such materials the temperature-dependent linear term in the specific heat is much larger ($7\text{--}50 \text{ erg g}^{-1} \text{ K}^{-2}$) than in the corresponding crystalline state, in which a linear term is negligibly small^{11,12}. Data presented here indicate that melanins also exhibit a large linear term ($50\text{--}200 \text{ erg g}^{-1} \text{ K}^{-2}$) and that they seem to undergo a phase transition as indicated by the heat capacity near 1.9 K . These effects occur in synthetic as well as natural melanins, but they are most pronounced in the melanosomes isolated from a human melanoma.

Table 1 Low temperature specific heat data for melanin samples

		γ (erg g ⁻¹ K ⁻²)	β (erg g ⁻¹ K ⁻⁴)	δ (erg g ⁻¹ K ⁻⁶)	T_0 (K)	ΔC at T_0 (erg g ⁻¹ K ⁻¹)
Melanin	1% H ₂ O	50 ± 9	120 ± 2	1.64 ± 0.09	1.87	940
Melanin	20% DEA	95 ± 9	99 ± 2	1.84 ± 0.09	1.92	770
Melanosomes	10% H ₂ O	220 ± 20	99 ± 4	2.0 ± 0.20	1.95	2,530

All values were determined by data points above approximately 2 K using the normal fitting equation $C = \gamma T + \beta T^3 + \delta T^5$.

Melanins can be prepared by the auto-oxidation of L-dopa⁵ and also by adding 20% (by weight) of diethylamine (DEA) to the starting mixture of L-dopa and twice-distilled water. Since the melanins exist in the cell as part of a cell organelle, the melanosome, a comparison of the properties of the intact melanosome with that of synthetic melanins was necessary to evaluate the function of the melanins in the intact organelle. The isolation of melanosomes for specific heat measurements was as outlined in Fig. 1. Human malignant melanoma cells obtained at autopsy were used.

Each calorimetric run involved a succession of heat inputs to the sample of a predetermined amount of calories, followed by measurement of temperature increases, using well-calibrated germanium thermometers. The measurements were repeated three times, particularly for the two synthetic melanins, to obtain enough data points for an accurate determination of the electronic specific heat coefficient. The data were fitted to the usual equation $C = \gamma T + \beta T^3 + \delta T^5$ by the least squares method. The results are shown in Fig. 1 and Table 1, in terms of C/T as a function of T^2 , where C is the specific heat in the units of g⁻¹ K⁻¹, and T is absolute temperature.

The values of the β coefficient in Table 1 indicate that the three materials are quite similar in their lattice contribution to the specific heat. The values of δ are also similar but considerably smaller. The linear term γ values, however are surprisingly large and show a consistent increase as the material

approaches the biologically relevant form. The smallest γ occurs in the highly synthetic melanin, which was polymerised in twice-distilled water. The melanin polymerised with diethylamines shows a higher γ value. (Amines were chosen since they are likely to be present in cellular conditions.) The highest γ value occurs in the biological extract from a human tumour. The increase in γ between the synthetic and the biological material is of clear interest. Although the reason for this is not understood at present, the close agreement in the phonon contributions for the different melanins suggests that the change in the linear term coefficient is unlikely to be due to a change in the phonon spectrum.

An interesting anomalous feature of the data is the observation of a discontinuity at about 1.9 K for all melanins measured in the present experiment. As can be seen in the inset to Fig. 1, this anomaly, though relatively small, is significant beyond the scatter of the data points. The shape of the anomaly seems to be different from that expected from an ordinary Schottky, or λ type, transition. Blois *et al.*⁸ measured electron spin resonance for both natural and synthetic melanins and showed the g -value to be close to 2, and a paramagnetic behaviour following the Curie law over a wide temperature range from 500 K down to 4.2 K. They concluded that the observed paramagnetism was due to unpaired electrons associated with free radicals in the amorphous melanin polymers, rather than an accidental contamination by some ferromagnetic impurities. The presence of

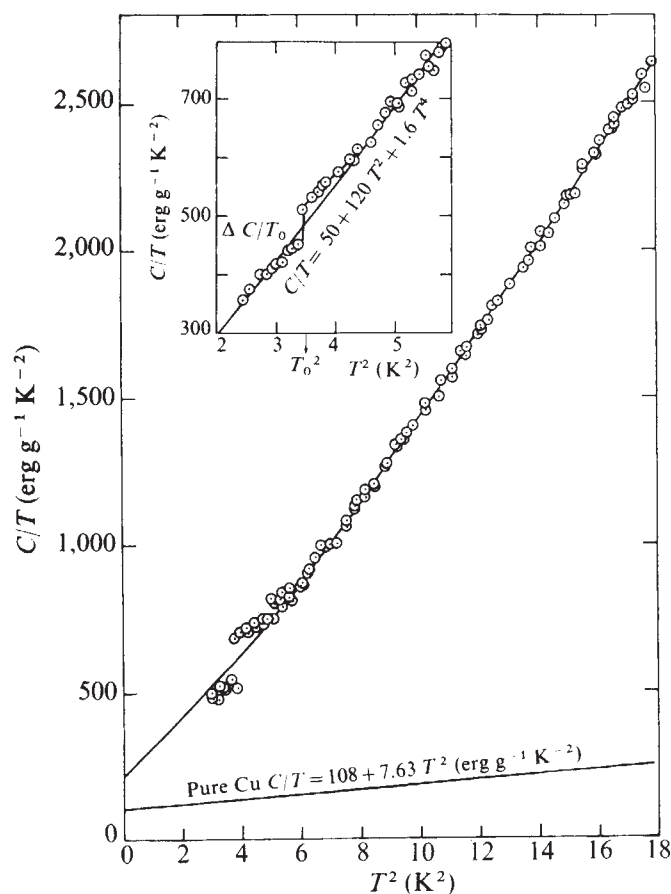


Fig. 1 The usual C/T against T^2 plot for the melanosome sample. The discontinuous change in the range of 1.9 K is shown for synthetic melanin in the inset. The corresponding plot for copper shows a similar value of the linear coefficient, but substantially different higher term coefficients. The tumour was dissected to remove connective tissue, passed through a fine mesh screen and then vortexed for 15 s in an isotonic salt solution containing 8% sucrose. Phase microscopy examination indicated that most cytoplasmic membranes were ruptured, but most nuclei remained intact. The suspension was centrifuged at 500g for 10 min to remove unlysed cells, cell nuclei and cell debris. The supernatant containing a mixture of cytoplasmic remnants and melanosomes, was spun at 8,000 r.p.m. in a Beckman SW27 Rotor for 20 min. The resultant supernatant was then discarded and the pellet resuspended in 5 ml of an 8% sucrose solution by sonification at about 50 W cm⁻¹ at 10⁴ cycles s⁻¹. The material was then layered on an exponential sucrose density gradient (5% sucrose at the top) and spun at 10⁴ r.p.m. for 20 min in an SW27 Rotor. The top two-thirds was then fractionated off and the remaining material resuspended and washed twice by centrifugation. The final pellets were found to contain about 3.5 g of material which, in the dehydrated state, was reduced to about 1.6 g, containing approximately 1% water. To prepare specimens suitable for calorimetric work, both synthetic melanins and melanosomes were compressed and cast into cylindrical moulds. After removal from the mould, a thermometer and heater wire were attached directly to each sample. Following the measurement, their contribution to the specific heat was corrected in the usual manner¹³. The DEA melanin sample was also made in the same way as above, but using a small amount of water. After the calorimetric experiment, this water was removed by heating in a vacuum and was found to constitute about 10% of the total weight of the sample. The weights of the synthetic melanins, melanosomes, and the DEA melanin samples were 2.30, 1.68 and 3.20 g respectively. The specific heat was measured in a semi-automatic calorimeter system in the ⁴He range in the usual manner^{13,14}.

the paramagnetism at 4.2 K strongly suggests that the specific heat data above the anomaly temperature to (~ 1.9 K) should also correspond to a paramagnetic state, since no specific heat anomaly is observed between 1.9 and 4.2 K. Thus, the anomaly at ~ 1.9 K is probably associated with a magnetic transition, possibly from paramagnetism to antiferromagnetism. The apparently good fit to the equation $C = \gamma T + \beta T^3$ of the few data points below 1.9 K may indeed be due to the presence of antiferromagnetism in the amorphous melanins. It is also possible that the large linear term is of magnetic origin rather than due solely to the amorphous structure. Antiferromagnetism in amorphous structures has been studied both experimentally and theoretically in amorphous glasses containing some transition elements^{15,16} where, however, no sharp magnetic transition was reported.

The melanosome has long been considered a passive cellular organelle. Its considered role as a photoprotective agent in the skin and other illuminated areas, could not explain its presence and function in the non-illuminated areas (for example, the midbrain). A single hypothesis was developed, on the basis of a quantum mechanical model of disordered materials (amorphous semiconductivity) to explain the functional role of the melanosome in both illuminated and non-illuminated areas^{2,4,7}. This hypothesis was based on electron-phonon interactions, which seems to be particularly strong in melanins, and on the large density of available energy states.

A particularly useful probe for determining the nature of these states is a measurement of low temperature specific heat. The measurements presented here include two anomalies, a transition and an unusually high linear contribution. The observed anomalies probably arise as a result of the electron-phonon coupling and high density of unpaired spins, which until now were difficult to correlate. Further experimental measurements at near the transition temperature may yield a detailed quantum mechanical description of the states, which will then afford a more precise understanding of the biological functions of melanosomes than has been possible to date.

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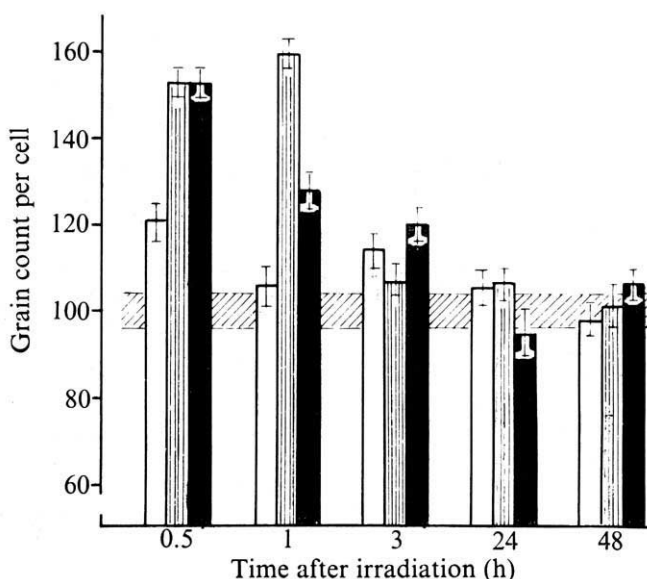
³H-concanavalin A binding of X-irradiated human fibroblasts

STUDIES on the lectin-binding capacity of plasma membranes form a valuable approach for the detection of various alterations in the glycoprotein components of membrane structures. In studying the effect of ionising radiation on the Golgi complex as one of the main structures for glycoprotein biosynthesis or assembly¹, we wanted to obtain quantitative data for the characterisation of the possible radiation-induced changes of plasma membrane glycoproteins. Using ³H-concanavalin A (³H-con A), specific lectin for glucose- and mannose-containing receptors², we observed a temporary change in the binding capacity of X-irradiated human fibroblasts.

Human WI38 fibroblasts were grown on coverslips in Bellco tubes with 10% heat-inactivated calf serum and 90% Parker solution containing penicillin (100 IU ml⁻¹) and streptomycin (100 µg ml⁻¹). Cultures were irradiated with a THX250 machine (200 kV, 20 mA, 0.5-mm copper filter, SSD 60 cm). Total absorbed doses were 90, 226 and 905 rad. After irradiation the medium was changed for an unirradiated one collected from cell cultures of the same age. At various times after irradiation the coverslips were washed three times with cold phosphate-buffered saline (PBS) solution and incubated for 5 min at 0 °C (ref. 3) with PBS containing ³H-con A (1 µCi ml⁻¹) (New England Nuclear, 60 Ci mM⁻¹). Unbound tritiated lectin was washed out five times from the coverslips with a cold PBS. Coverslips were fixed in Carnoy solution, coated with liquid emulsion by the dipping method (Ilford Nuclear Research Emulsion, Type G5), exposed for 5 d at 4 °C, and fixed and stained with 5% Giemsa solution. Grains were counted in 100 cells for each dose and time point.

In the conditions specified above the number of grains developed in cells were counted. Irradiation resulted in an early (up to 30-60 min) and temporary increase in the amount of bound lectin at the doses applied; the amount of lectin bound to the plasma membrane returned to control level (Fig. 1). Maximum increase was about 60%

Fig. 1 Dose- and time-dependent changes in grain numbers of X-irradiated WI38 human fibroblasts labelled with ³H-con A. Bars represent s.e.m. Diagonal hatching, control level; open columns, after 90 rad; vertically hatched columns, after 226 rad; solid columns, after 905 rad.



of control. Similar results were obtained when we considered radioactivity per cell, using the liquid scintillation method with labelled cells.

Our result may provide a new way of detecting early structural and functional changes in the plasma membranes because of radiation treatment. The temporary disturbances in membranes which can be detected by this method may lead to elucidation of other phenomena. Further investigations using different labelling conditions for concanavalin and other lectins may also provide promising radiobiological methods or practical tests for the early detection of the extent of radiation injury in animals or humans using their lectin-binding capacities, for example, blood cells. A similar increase in ^{125}I -con A binding *in vitro* by irradiated (500 rad) porcine lymphocytes was detected after 30 min. Further experiments are in progress to measure the lectin binding capacity *in vivo* and *in vitro* of irradiated blood cells using autoradiographic and liquid scintillation methods.

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Detection of bound ferulic acid in cell walls of the Gramineae by ultraviolet fluorescence microscopy

THE phenolic acids, ferulic and *p*-coumaric, are bound to cell walls of plants of the Gramineae (which include grasses and cereals) and it was thought that these acids were esterified to lignin and so occurred only in lignified cell walls¹⁻⁴. But it has been shown recently that in cell walls of *Lolium multiflorum* Lam., which contain ferulic acid and a small amount of *p*-coumaric acid mainly as their *trans* isomers, the acids are esterified at their carboxyl groups to polysaccharides⁵⁻⁷. The digestibility of grasses by ruminants can be predicted by estimating the amounts of the acids released from the plant cell walls after treatment with a commercial cellulase preparation⁸. The acids could be important in influencing the degradation of plant organic matter in the rumen or in soil and may act as growth inhibitors of plant pathogens⁹. We now report results obtained by ultraviolet fluorescence microscopy, which indicate that ferulic acid is bound to polysaccharides of the walls of a wide variety of cell types of the Gramineae; these walls give a negative phloroglucinol-HCl test for lignin¹⁰.

Phenolic acids and their ester derivatives often show changes in fluorescence intensity and colour in ultraviolet radiation when treated with ammonia¹¹. Ultraviolet fluorescence microscopy of transverse sections (mounted in glass distilled water, pH 5.4) from the middle of an immature internode of *Lolium temulentum* L. showed that all the walls fluoresced blue. This fluorescence was intensified by exposure to ammonia vapour or treatment with 0.1 M ammonium hydroxide solution (pH 10.3), which caused the fluorescence colour of the walls of many cell types to change to green (Fig. 1 and Table 1). This change was dependent on pH and reversed by 0.1 M sodium acetate buffer, pH 4.0. A similar change was shown by carbohydrate esters of ferulic acid obtained from ryegrass cell walls and separated by thin-layer chromatography^{3,6},

suggesting that the walls which fluoresced green contained ferulic acid units esterified to polysaccharides.

Further evidence that the green fluorescence of the cell walls is associated with ferulic acid was obtained by treating sections of immature internode with 1.0 M sodium hydroxide solution at 20 °C for 16 h, which removes esterified phenolic acids from cell walls¹⁻⁴. Cells which previously fluoresced green showed little fluorescence after treatment.

In addition to a negative phloroglucinol-HCl test, the absence of lignin from the walls of most cell types which fluoresced green was indicated by their complete degradation by a crude cellulase (Fig. 2). But the walls of the sub-epidermal sclerenchyma, although they fluoresced green and gave a negative phloroglucinol HCl test, were not degraded by cellulase, apparently because they were partially lignified.

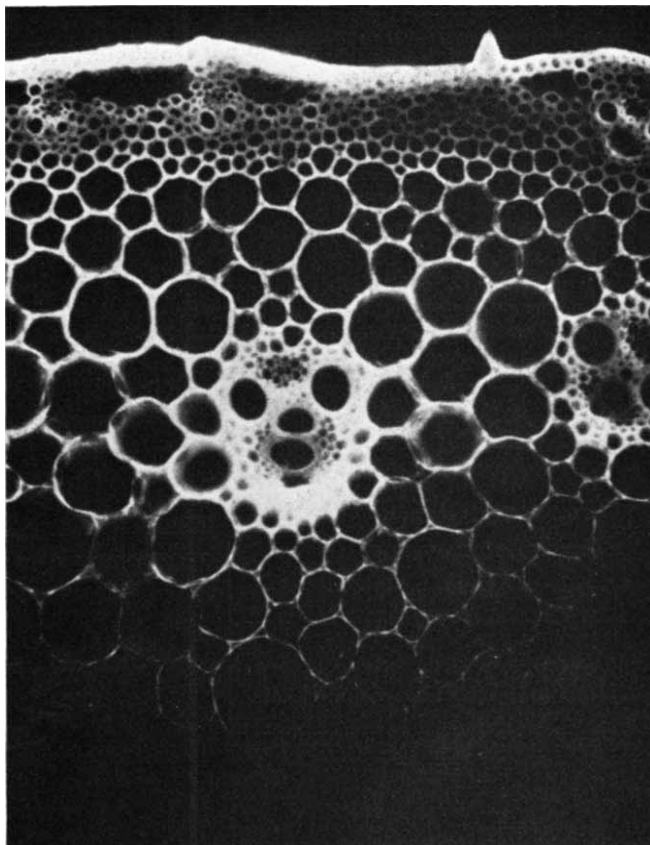


Fig. 1 Transverse section (100 μm thick) from the middle of an immature internode 11.8 cm long (internode below point of insertion of flag leaf) of *Lolium temulentum* L. cut under water using a Vibratome (Oxford Laboratories, San Mateo, California) and mounted in 0.1 M ammonium hydroxide. Photograph taken on a Zeiss Large Universal Fluorescence Microscope (HBO 200 Hg vapour lamp, UGI, exciter and No. 41 barrier filters). Plants were grown in controlled environmental conditions (day 23 °C, night 18 °C, light intensity 130 W m^{-2} , photoperiod 16 h). ($\times 168$.)

Cellulase did not alter the fluorescence behaviour of the walls of the cell types which were not degraded. Contrary to earlier suggestions¹², these observations show that blue fluorescence in ultraviolet radiation does not necessarily indicate the presence of lignin. But those walls which remained blue after treatment with ammonia, and gave a positive phloroglucinol-HCl test, were not degraded by cellulase, which suggested that they were lignified. Some esterified ferulic acid may also have been present in such walls, its green fluorescence being masked by the blue fluorescence of the lignin.

Cell walls of other parts of *L. temulentum* showed fluorescence behaviour similar to that of the immature internode (Table 1). Usually those walls which continued to fluoresce blue after treatment with ammonia gave a positive

Table 1 Fluorescence colour of *Lolium temulentum* cell walls of different cell types after treatment with ammonia

Plant part (transverse section)	Blue	Green
Immature stem	Xylem vessels, xylem tracheids and bundle sheath	Xylem parenchyma, phloem, sclerenchyma, epidermis, parenchyma and chlorenchyma
Fully elongated stem	Xylem vessels, xylem tracheids, bundle sheath, sclerenchyma, epidermis and parenchyma (adjacent to sclerenchyma)	Xylem parenchyma, phloem, parenchyma (adjacent to central cavity) and chlorenchyma
Leaf lamina	Xylem vessels, xylem tracheids and inner bundle sheath	Xylem parenchyma, phloem, outer bundle sheath, sclerenchyma (blue middle lamella), epidermis and mesophyll
Leaf sheath	Xylem vessels, xylem tracheids, inner bundle sheath, sclerenchyma and upper epidermis	Xylem parenchyma, phloem, outer bundle sheath, lower epidermis, parenchyma and mesophyll
Glume	Xylem vessels, xylem tracheids, bundle sheath, sclerenchyma, epidermis and parenchyma (adjacent to sclerenchyma)	Phloem, parenchyma (adjacent to chlorenchyma) and chlorenchyma
Lemma	Xylem vessels, bundle sheath, sclerenchyma and outer epidermis	Phloem, inner epidermis and chlorenchyma
Palea	Xylem vessels, inner bundle sheath, sclerenchyma and outer epidermis	Phloem, outer bundle sheath, inner epidermis, parenchyma and chlorenchyma
Root	Xylem vessels, stele fibres, endodermis and pericycle	Phloem, epidermis, cortical parenchyma, root hairs and exodermis
Mature grain	Outer thick walled cells of pericarp	Inner thin walled cells of pericarp, nucellus, aleurone layer and starch cells of endosperm
Coleoptile	Xylem vessels	Phloem, bundle sheath, inner and outer epidermis and parenchyma
Ovary (squash preparation)	No cell types	All cell types
Stamen (squash preparation)	No cell types	All cell types
Pollen (mechanically isolated walls)	Walls of grains	No cell types

test with phloroglucinol-HCl, whereas those that fluoresced green did not. Pollen cell walls, however, although negative to phloroglucinol-HCl, fluoresced blue after treatment with ammonia, probably because of the presence of sporopollenin¹³.

Ferulic acid bound to unlignified cell walls seems to be widespread in the Gramineae. This was indicated by a fluorescence behaviour similar to that of *L. temulentum* when transverse sections of the following grasses (leaf laminae) and cereals (immature internodes) were used: *Dactylis glomerata* L. cv. S37, *Festuca arundinacea* Schreb. cv. S170, *Festuca pratensis* Huds. cv. Comtesa, *Lolium multiflorum* Lam. cv. S22, *Lolium perenne* L. cv. S24 and *Phleum pratense* L. cv. S48, *Avena sativa* L., *Hordeum vulgare* L., *Triticum aestivum* L. and *Zea mays* L. cv. Caldera 535.

In contrast to the situation with the Gramineae, ferulic and *p*-coumaric acids or their carbohydrate esters were not released in detectable amounts after treatment of cell walls isolated from young petioles of *Brassica oleracea* L. var. *acephala* DC. cv. Maris Kestrel (Cruciferae) with sodium hydroxide⁴ or cellulase⁸. The fluorescence behaviour of the cell walls of a young petiole of this plant was consistent with the absence of ferulic acid. Only the cuticle and cell walls of the xylem fluoresced in ultraviolet radiation and

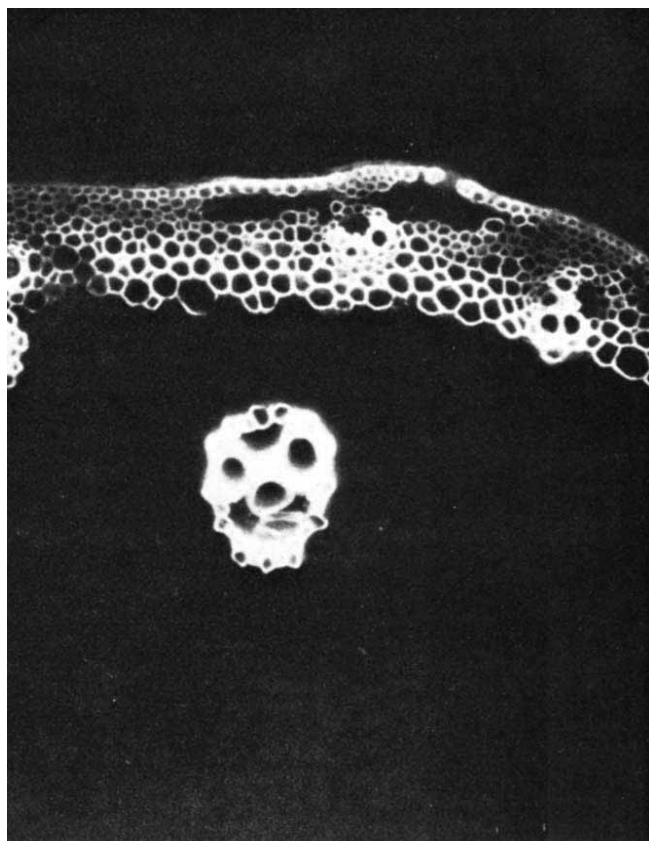


Fig. 2 As Fig. 1, but section treated with a solution of crude commercial cellulase (*Oxyporus* sp., Merck, 2.5 mg ml⁻¹ 0.2 M NaOH-AcOH buffer, pH 4.8 containing 0.02% NaN₃) for 16 h at 37 °C and washed with water. ($\times 168$.)

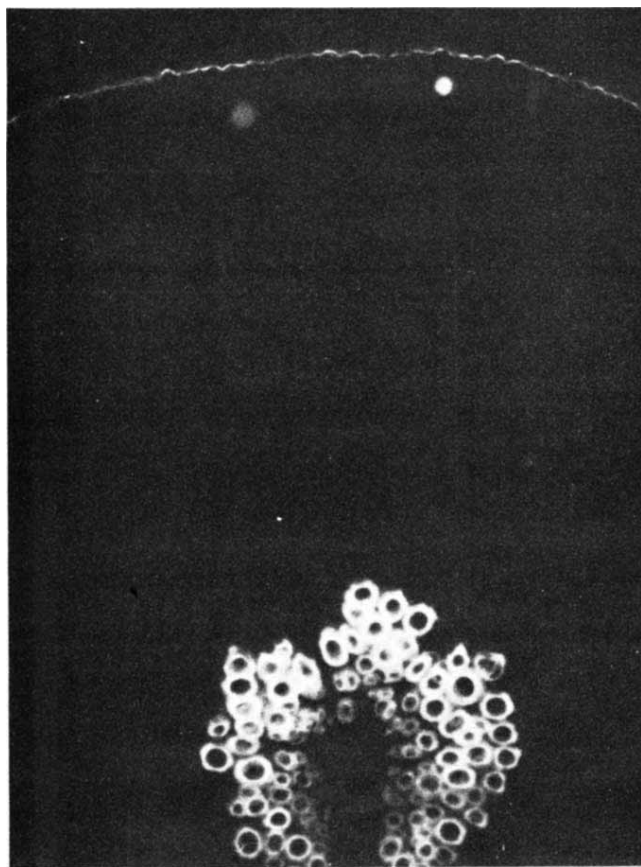


Fig. 3 Transverse section (70 μm thick) of young petiole of field-grown kale. Cut, mounted and photographed as described in Fig. 1. ($\times 168$.)

the fluorescence remained blue after treatment with ammonia (Fig. 3). Cell walls of *Trifolium pratense* L. cv. Hungaropoly and *Onobrychis viciifolia* Scop. cv. Cotswold Common (Leguminosae) showed similar fluorescence behaviour, indicating the absence of ferulic acid.

The synthesis and secretion of polysaccharides in the slime of the root cap is analogous to the synthesis and deposition of the matrix polysaccharides in cell walls. The Golgi apparatus and its associated vesicles are considered to be the site of synthesis and transport of the polysaccharides of the root-cap slime, and are believed to have a similar role in the case of the cell-wall matrix polysaccharides¹⁴. The root-cap slime of *Z. mays* showed blue fluorescence which changed to green after treatment with ammonia, suggesting the presence of ferulic acid esterified to the polysaccharides. It is possible that the site of bonding between ferulic acid and the polysaccharides of slime or of the cell-wall matrix is the Golgi cisternae, the resulting esters being transported within the associated vesicles.

Lignin could be synthesised in the Gramineae by oxidative coupling reactions involving ferulic acid units which are esterified to wall polysaccharides. This is consistent with the finding that the walls of the sub-epidermal sclerenchyma cells and the adjacent parenchyma cells of the fully elongated internode of *L. temulentum* fluoresced blue after treatment with ammonia, in contrast to the green fluorescence of the walls of similar cells of the immature internode (Table 1). Although lignified walls may contain some unpolymerised ferulic acid esterified to polysaccharides, we have shown that as stems of *L. multiflorum* mature, less water-soluble esters are released from their walls by treatment with cellulase.

The work reported here leads us to question conclusions drawn when radioactive lignin precursors were fed to

graminaceous species and the radioactivity detected in the cell walls was attributed to lignin¹. We suggest that at least some radioactivity is associated with phenolic acids esterified to non-lignified walls. This suggestion is supported by a report that after feeding ¹⁴C-cinnamic acid to wheat coleoptiles, radioactive ferulic acid was released from the walls by sodium hydroxide¹⁵. The production of vanillin or *p*-hydroxybenzaldehyde by nitrobenzene oxidation of cell walls has been used as a test for lignin¹ but these compounds could be obtained from ferulic acid and *p*-coumaric acids respectively.

We thank Drs L. H. P. Jones and E. L. Leafe for their interest in this work.

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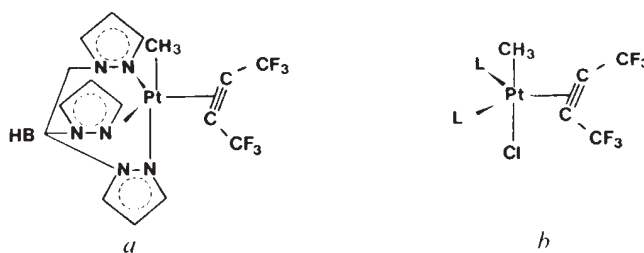
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Corrigenda

In the article "Mechanism of catalysis of hydrocarbon reactions by platinum surfaces" by G. A. Somorjai and D. W. Blakely (*Nature*, **258**, 580; 1975), the sentence which begins in line 26 of the second column on p. 580 should read . . . The rate of *n*-hexane production per kink site is determined by the slope of the line in Fig. 3b, which gives 4×10^{-28} mol s⁻¹ per kink atom. This is almost an order of magnitude higher than the slope in Fig. 3a which corresponds to 5×10^{-29} mol s⁻¹ per step atom.

The sentence which begins in line 10 of the caption to Fig. 3 should read . . . The rates of hydrogenolysis per surface site at the slope of the lines representing hydrogenolysis are 4×10^{-28} mol s⁻¹ per kink atom and 5×10^{-29} mol s⁻¹ per step atom.

In the article "Mechanism of reactions at square-planar metal centres" by F. R. Hartley and J. J. Périé (*Nature*, **256**, 636; 1975) Fig. 1a and b are incorrect. The correct forms are reprinted below.



reviews

Worthy of sponsored pilgrimage

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Johannes Kepler (1571–1630), an engraving from *Great Astronomers* by Robert Ball (London, 1895).

CENTENARY celebrations of the births and deaths of illustrious men are usually prompted more by an urge to conviviality than by a feeling that history ought to repeat itself at 100-year intervals. Just as it seems to be a law of economics that the rich grow richer as the poor grow poorer, so the great tend to grow greater at the expense of those who are not judged worthy of sponsored pilgrimage and fanfare. The resulting distortion of history will be kept to a minimum only if we stick to a high enough 'fame threshold'—and if Kepler is to fall below our threshold, who will be above it? A volume like that* now edited by Arthur and Peter Beer, with more than 1,000 pages and encompassing more than 130 contributions, must inevitably include its share of empty eulogy, but the bibliographical advantages of having so much Kepler material in one place are very great—almost as great, in fact, as the difficulty in reviewing it adequately.

The volume takes in the full texts (in English translation where necessary) of the symposia held 400 years after Kepler's birth, in 1971, at Philadelphia, Leningrad, Graz, Linz,

London, Paris, Berlin and Evanston. There are also summaries of other papers, as well as a number of specially written articles published for the first time. The book is richly illustrated—and only Plate 6.4 needs to be read in a mirror. The translations, mostly by Judith Field and Richard Rodman, cast doubt on the editors' plea that translations, like women, are unfaithful when beautiful and not beautiful when faithful. There are 20 sections to the volume, but they fall into four broad groups: Kepler's intellectual influence on contemporaries; studies of his religious, philosophical, alchemical and mystical beliefs; mathematical and physical theories; and other matters which occupied Kepler's thoughts. At the end of the book there are extremely useful bibliographical and iconographic supplements and indices. Altogether, the volume is a monument to the industry and editorial logic of the Beers, father and son.

The short note by D. T. Whiteside, on Kepler's ovals, is an *apéritif* for the invaluable enlarged version of his paper which has since appeared in *Journal for the History of Astronomy*. Another note by Otto Neugebauer, shows that it is hardly surprising to find contemporaries of Kepler failing to understand the import of his work, faced as they were with "rather trivial obstacles . . . on practically every page". The obstacles in question are the trivial computing errors, unexplained changes in parameters, references to (Tycho Brahe's) observations accessible to scarcely anyone else, and so forth. To prove the point in an utterly convincing way, Neugebauer takes the penultimate chapter of *Astronomia Nova*, wherein Kepler shows the need for an elliptical orbit and an equal-area rule.

The *Astronomia Nova* was written in the form of a self-critical intellectual autobiography, and has generally been regarded as an honest one. But as instances of papers showing the circuitous, not to say devious, nature of the path travelled by Kepler's thoughts, one may cite those by Owen Gingerich and E. J. Aiton (three each), as well as that by C. A. Wilson. Gingerich, who has written on the same material extensively elsewhere, makes some use of Kepler's unpublished notebooks (now in Leningrad) on Mars. He emphasises

the importance to Kepler of a three-dimensional approach, and aptly suggests that the idea that the lines of planetary apsides (and hence the planetary planes) should pass through the Sun is important enough to be called Kepler's zeroth law. (The zeroth law is of course logically, if not historically, derivable from the first.) A problem often touched on but rarely tackled with much fervour is that of the interplay of observations (potential and original) and conjecture in Kepler's work. Could the ellipse be observationally distinguished from the alternatives, for example? Curtis Wilson's contribution begins with Newton's assessment: "Kepler knew the orb to be not circular but oval and guessed it to be elliptical". Wilson's all too brief paper explains very clearly in what sense Kepler was indeed guessing. One of the most thorough of recent studies of Kepler's accuracy—in connection with the solar theory—is that reported here in a shortened version of an admirable paper by Y. Maeyama.

Needless to say, Newton was not primarily concerned with Kepler's path to his discoveries. With his "Kepler's Century: Prelude to Newton's", I. B. Cohen starts the volume with, as it were, a Newtonian justification for Kepler's existence, not to mention some entertaining material on later attitudes to the Kepler story.

Outside astronomy proper, the volume includes a number of invaluable sketches which will be of lasting interest to scholars. Jürgen Hübner's assessment of Kepler's theology, J. O. Fleckenstein's of the neoplatonic movement, and Gérard Simon's account of his attempted reform of astrology, for example, help to explain the Kepler who could write (to Mästlin in 1598): "I am a Lutheran astrologer, throwing out the chaff and keeping the grain". Karin Figala considers Kepler's alchemy in the light of a recently discovered funeral oration for the Emperor's personal physician (Martin

**Kepler: Four Hundred Years*. (Proceedings of conferences held in honour of Johannes Kepler. Vistas in Astronomy, Volume 18). Pp. xix + 1,034. (Pergamon: Oxford and New York, 1975). Subscribers' price: \$120.00, £50.00. Non-subscribers: \$150.00, £63.00. Available with Volume 17 on Copernicus, Two-volume subscribers' price: \$140.00, £60.00. Non-subscribers: \$175.00, £75.00.

Ryland II), with a poem by Kepler. This contribution sets higher standards of scholarship than some of the astronomical items. The short papers on Kepler's wanderings from Württemberg to Graz, to Prague, and so on, would make on their own a most enjoyable Baedekker's guide to the manners no less than to the places of Kepler's time.

One of the most surprising things about the 1971 symposia is that so few historians took the opportunity of adding to our understanding of Kepler's optics. A précis of J. A. Lohne's study of Kepler, Harriot, and the sine law is worth noticing, and yet for the full text one must turn to the *Weil der Stadt* proceedings. That a similar remark must be made for many of the articles in this volume is not a weakness but a source of strength. This is

essentially a 'State of the Art' report. In a report within a report, Robert S. Westman considers two early historiographical traditions in Kepler scholarship. In one of these, the history of science was regarded as a useful repository of still valued scientific ideas. The second, hermeneutical, tradition entailed an investigation of the philosophical foundations of Kepler's discoveries. Westman, speaking in Philadelphia towards the end of 1971, placed those papers given to the European conferences against this double historiographical background. This impressive and important volume, in an almost uniformly important series, is therefore complete to a fault, containing as it does its own review. It will be consulted long after its weighty (3 kg) contents have parted company with its covers.

J. D. North

Finite geometries and designs

Graph Theory, Coding Theory and Block Designs. (London Mathematical Society Lecture Note Series, No. 19.) By P. J. Cameron and J. H. van Lint. Pp. v+114. (Cambridge University, Cambridge, London and New York, September 1975.) £2.80.

THIS splicing of lecture notes from two courses of seminar lectures given at Westfield College in 1973 is intended for a specialised group of mathematicians, combinatorialists interested in finite geometries and designs, and serves to introduce them to those parts of graph theory and algebraic coding theory that have recently interacted fruitfully with design theory. The authors have, however, provided a brief introduction to design theory proving certain elementary results, stating certain deeper results, and describing many of those designs that arise geometrically. Thus, in principle, the book can be read by any mathematician.

Chapters 2-5 are concerned with the connections between design theory and strongly regular graphs. Chapter 6 is a brief survey of known results concerning so-called "two graphs", a combinatorial structure that arises naturally in the study of doubly-transitive permutation groups. The remaining nine chapters are devoted to those aspects of algebraic coding theory of interest to design theorists. Chapters 9-11 constitute an introduction to coding theory and chapter 15, the last, an introduction to association schemes (a generalisation of strongly regular graphs) and some of the recent work of Delsarte. Sandwiched between are five chapters concerned with those codes of greatest combinatorial interest: Reed-Muller codes, self-orthogonal codes, quadratic-residue codes, symmetry codes, nearly perfect and uniformly packed codes.

The expert will note that proposition 13.4 is incorrect without an added hypothesis and that the remark on page 69 is false since ovals are always seen in the dual. In spite of minor flaws and sometimes sketchy proofs this slender volume should prove useful to its intended audience.

E. F. Assmus, Jr

Applying a technique

Mössbauer Spectroscopy. (Topics in Applied Physics, Vol. 5.) Edited by U. Gonser. Pp. xviii+241. (Springer: Berlin and New York, 1975.) DM70; \$30.10.

THE increasing use of the Mössbauer technique during the past 15 years in fields as diverse as relativity and archaeology, resulted in the publication of about 1,200 research papers in 1974. Its use in conjunction with more standard techniques in, for instance, chemistry and metallurgy departments means that it is no longer practicable to produce a book giving a critical study of the whole range of applications. Several good introductions to the basic principles exist already but the purpose of the present volume is both to introduce the technique to non-specialists and also to present them with general information on the applications related to their own fields of interest. Thus the introductory chapter is followed by five chapters, written by different authors, concerned with applications in chemistry, magnetism, biology, lunar geology and mineralogy and physical metallurgy. The level seems to be aimed at that of the research worker although the treatment varies from chapter to chapter, with those concerned with chemistry and magnetism requiring familiarity with the methods of quantum mechanics.

In the introduction, the conditions required for the emission of Mössbauer γ rays are described together with the properties of the hyperfine interactions, between the nucleus and its electronic environment, which form the basis of a Mössbauer measurement. A short experimental section is marred by poor

quality spectra and a misleading comparison of γ -ray detectors. Two chapters are devoted to a discussion of the information obtained from the hyperfine interactions, concerning the electronic structure and bonding properties in chemical compounds in general, and in metal proteins. In the latter case the potentially valuable use of the technique to determine the state of the iron in clinical specimens of blood or tissue is mentioned, with the hope that this could lead to advances in medical diagnosis. In the chapter on magnetism there is no mention of work on metals and alloys, and the text is concerned largely with studies of spin orientations in mixed iron oxides. That chapter, particularly, suffers somewhat from being restricted to the isotope iron-57.

The use of the Mössbauer effect as an analytical tool to determine molecular composition is brought out in the chapter on lunar geology and mineralogy, though little discussion is given of the main findings of other measurements. The final chapter on physical metallurgy seems to be at about the right level for an introductory survey. Methods are given for the analysis of the spectra of alloys and intermetallic compounds, with many examples including the study of carbon atoms in steels, the invar problem and the structure of amorphous alloys.

Although the level of presentation varies, in general it is higher than that usually found in an introduction for the non-specialist. As such, the book is more likely to be read by the Mössbauer specialist looking for a presentation of applications outside his own field of study. More comprehensive surveys in each field exist already and the price of \$30.10 is rather high for the purchase of a personal copy.

G. Longworth

Erratum. The size of the Mayan bas relief shown on p349 of the reviews section (*Nature*, 259, January 29, 1976) was incorrectly stated as 1.3×1.3 cm. The correct size is 1.3×1.3 m.

Atmospheres and other fluids

Atmospheric Thermodynamics. (Geophysics and Astrophysics Monographs: an International Series of Fundamental Textbooks, Vol. 6.) By J. V. Iribarne and W. L. Godson. Pp. x+222. (Reidel: Dordrecht and Boston, Massachusetts, 1973.) n.p. *Advanced Fluid Mechanics: An Introduction.* By A. J. Raudkivi and R. A. Callander. Pp. xii+325. (Edward Arnold: London, July 1975.) Boards £12.00; paper £5.00.

THE book by Iribarne and Godson, which is based upon undergraduate lecture courses given by the authors, will be welcomed by students of the atmospheric sciences. It is one of the few textbooks in meteorology to deal exclusively with atmospheric thermodynamics and doubtless it will soon become deservedly established as a standard recommended text. The authors assume some knowledge of basic thermodynamics but devote the first four chapters of the book to a review of the fundamental relationships and concepts in the subject. There is some reference to the applicability of these relationships to meteorological problems in the introductory chapters but that aspect is more strongly emphasised in the latter half of the book. Strictly thermodynamic atmospheric processes are discussed at length (fog formation, cloud physics, precipitation, and so on) but sections are also included in which the importance of atmospheric dynamics and radiative processes is emphasised.

Throughout the text one is impressed by the concise and systematic treatment of the subject. Problems are given at the end of each chapter, the diagrams are informative and the book as a whole is well written and well presented. It has a good balance between basic thermodynamics and meteorological applications and will be useful to students and research workers alike.

With students of engineering particularly in mind, Raudkivi and Callander in their book have attempted to produce a work filling a gap which they feel exists between 'elementary' and 'advanced' texts on fluid mechanics. Claiming that the latter have been written almost exclusively by applied mathematicians (Batchelor's "Introduction to Fluid Dynamics" is cited as one example) their primary requirement has been to make the mathematical background simpler and more accessible to students already familiar with elementary fluid mechanics. It will be difficult for the reader to discern any significant improvement upon Batchelor's book in this regard. If indeed such a comparison is appropriate.

A lengthy section on turbulent flows is included, for example, as is an interesting survey of diffusion and dispersion. The remainder of the text is taken up with chapters on potential flow theory, boundary layers (surprisingly appearing after the section on turbulent flow) and fundamental relationships.

The latter contains much information (rheological models, tides) not usually included in such a section and, as with the rest of the book, it is well written and well presented. Particularly attractive features of the book are the inclusion of experimental data to complement theoretical ideas, and a generous supply of worked examples.

Of necessity, the authors have had to be selective in their choice of topics but they have produced a book which will be of interest to workers in several branches of fluid mechanics.

P. A. DAVIES

Basic astronomy

Astronomy Today. By Fred Hoyle. Pp. 179. (Heinemann Educational: London, October 1975.) £4.50.

Astronomy Today is a straightforward basic astronomy text, of the kind which many astronomers could write but which inevitably will reach a larger audience than most through the magic of the author's name. The book is lavishly illustrated and the text is clear, informative and readable. But given its title this is a disappointing book, which describes the Solar System in five chapters, the stars and life in the Universe in one each, and devotes the last chapter to Galaxies and the Universe, presenting a lucid exposition of Hoyle's picture of the redshift as indicating a Universe in which the mass of atoms increases with time.

This presentation of unorthodox ideas in such a way at the end of what is otherwise an orthodox book published in an educational series is curious, to say the least, and it seems that the UK publishers must take the blame for this, since the US edition of the book bears the much more apposite title *Highlights in Astronomy* (Freeman, 1975). The implication that this theory is as well established and broadly accepted as such fundamentals as the causes of seasons and eclipses is most ill-judged in the context of an educational text for young people. And the publishers are further guilty of misjudgement in describing the book on the cover as "ranging from the geological history of the Earth to the latest discoveries about neutron stars and black holes". Black holes are mentioned (once), but only in passing, and there is no mention at all of the state of the art in X-ray astronomy today,

the field in which most of the latest discoveries about black holes and neutron stars have been made.

This is a fine description of "the Solar System today", but in no way is the book representative of the exciting recent advances in high energy astrophysics that the cover and the author's name lead us to expect. It is doubly ironic that not only is Hoyle ideally equipped to write the book we are led to expect, but also that his exposition of novel cosmological ideas would sit far more happily in such a volume.

John Gribbin

Riboflavin

Riboflavin. Edited by Richard S. Rivlin. Pp. xiii+433. (Plenum: New York and London, 1975.) \$46.90.

THIS book comprises a collection of review articles by different workers on the chemistry, physiology and metabolism of riboflavin and the effects of riboflavin deficiency principally in man and rat. Hormonal regulation of riboflavin metabolism, studies on analogues and antagonists and a useful section on methods of assay are well described. Of special interest are the sections on the teratological consequences of riboflavin deficiency and the effect of deficiency on the growth of neoplastic tissue. Editorially the texts have been well cross-referenced and repetition has been minimised without adversely affecting content and readability.

Some abbreviations are used without a preceding definition—M:E ratio and PAH—and inconsistencies occur with others—for example, nicotinamide adenine dinucleotide, NAD (p162), DPN (p308). One section presents arguments for the further enrichment of bread as a means of improving riboflavin status in the US but alternative methods are hardly discussed.

The most serious omission is the lack of adequate detail and discussion on obtaining riboflavin-deficient rats. Chapter 10 mentions briefly (7 lines) the many factors that can influence the deficient state (p305) but without any explanation; and, for the diet used by the author, the reader is referred to a 'recent' reference 1951! This is presumably a mistake, as the diet to which one is referred in no way satisfies the 'severe criteria of deficiency' which the writer considers necessary.

In general, however, the book is well referenced and will provide a valuable and timely addition to the bookshelves of medical schools and institutions of nutrition and pharmacy.

David I. Thurnham

High fashion in liquid dielectrics

Simple Dielectric Liquids: Mobility, Conduction and Breakdown. By T. J. Gallagher. Pp. vi+154. (Clarendon: Oxford; Oxford University: London, October 1975.) £6.00 net.

"BREAKDOWN in liquids could readily become a compendium of unrelated observations", wrote Whitehead in the introduction to his book on breakdown in solids. Although this pronouncement was made from the cosiness of one of those periods when that subject seemed to support an established orthodoxy, it has always held more than a grain of truth. It is the tendency to try to create an orthodoxy which has acted as an impediment to progress in all of liquids research. Arguably, such periods of stasis are little more than a state of mind among referees for scientific journals and other influential workers. The result in a notoriously difficult experimental field is a wild veering of fashion more reminiscent of *haute couture* than scientific progress. Everything in liquid dielectrics has been ascribed successively to ions, electrons, molecular bonds, bubbles or particles, according to vogue.

Technologically this is a very important field. In power distribution alone a small improvement in electric strength would produce enormous financial economies, and in electronics the liquid state has not begun to offer the possibilities provided by the other states of matter. It is an ideal field for PhD training, being generally rather too speculative for industry, yet sufficiently demanding. There has been a large volume of published work which does not seem to be matched by a corresponding degree of solid achievement. Without doubt much of this is due to the nature of the test object itself. The most critical aspect of the dielectric sample, the metal-liquid interface, is almost a completely unknown quantity, yet it determines the transfer of charge in conduction experiments and also the probability of breakdown. It must therefore be disheartening for the new researcher to find a turgid literature rife with ill-controlled experiments and glib theories.

What can one say of an author who has the temerity to attempt to survey such a field? Gallagher has done as good a job as could possibly be expected. This review should rapidly become the *vade-mecum* for all researchers in the field, new or old. It is particularly stimulating to be able to pick out the high points in which one result condemned great chunks of the preceding literature; for example the discovery of the oxygen effect in breakdown, the e.h.d. stability theory, fast carriers in

pure liquids, the statistical nature of breakdown, and so on.

If one has to find the conventional adverse criticism, it is that the author maintains his excellent disinterestedness to a fault. Having made a remark which invalidates a theory (for example, that simple equilibrium thermodynamics cannot be applied to a transient irreversible process such as breakdown) he proceeds to give it a full treatment at the expense of others which might deserve more of his limited space. A similar remark applies to some of the experimental results (for example, the use of strange techniques of statistical selection possibly inspired by vision of the Holy Grail of Intrinsic Strength). These are, however, quibbles in respect of a text which deserves to become mandatory for any serious worker in the field. The diagrams, tables, bibliography, author and subject indexes are all excellent. Readers should note that the treatment does not include the proceedings of the most recent (1975) conference, which is published by the Delft University Press. **J. E. Brignell**

Coding for isoenzymes and haemoglobins

Haemoglobin, Isoenzymes and Tissue Differentiation. (North-Holland Research Monographs: Frontiers of Biology, Volume 42.) By C. J. Masters and R. S. Holmes. Pp. xiii+308. (North-Holland: Amsterdam and Oxford; Elsevier Scientific: New York, 1975.) Dfl.79; \$32.95.

THE isozyme (isoenzyme) concept, initiated by Markert and Möller 16 years ago, and the accompanying techniques of zone electrophoresis and specific histochemical staining, have led to new insights in many areas of biology and biochemistry. Essentially all enzymes are capable of existing in multiple molecular forms—isozymes—whether they are encoded in different genetic loci (multiple locus isozymes) or encoded in different alleles at a locus (allelic isozymes=allozymes). This book reviews primarily those isozymes (and proteins with specific function, for example, haemoglobins) which are encoded in different but related genetic loci. Two approaches are successfully used in presenting the concepts. The first is a discussion of the molecular, genetic, and evolutionary bases of the more thoroughly documented isozyme systems. The second is the illustration of some of the ways that isozymes can be used as tools to analyse the mechanisms of genetics, physiology, development and evolution.

This is not an exhaustive com-

pendium of all isozyme systems but selectively focuses on those areas of biology (cellular, developmental, genetic and evolutionary) and those enzymes, which represent the authors' areas of expertise. The results of key papers have been summarised (at times critically evaluated) and integrated into a broad contemporary biological context. The condensations of the material, the selected areas reviewed, and the thorough coverage of the earlier literature make this book a useful adjunct to the recently published four-volume proceedings of the Third International Isozyme Conference (Academic, New York) which is a comprehensive collection of original research reports covering almost all areas of biology.

The biological and experimental utility of isozymes is effectively and persuasively presented. The title is somewhat misleading, however, in that it fails to convey the evolutionary emphasis in the first third of the book. This section deals with the evolution of these multilocus isozymes (and haemoglobins), mechanisms of gene duplication, and the utilisation of these isozyme systems to establish phylogenetic relationships. A physiological rationale is often brought forward to explain the divergence in kinetic, physical and other properties of the isozymes as well as the divergence in the developmental and cellular specificity of their syntheses. There is, throughout the book, an excellent blend of evolutionary and developmental concepts. The middle third of the book analyses the differential expression of related genetic loci during development and the physiological relevance of their temporally and spatially specific syntheses.

The last third of the book is devoted to some current problems in isozymology. An interesting analysis is provided of the mechanisms involved in the regulation of isozyme levels, particularly the balanced contributions of preferential synthesis and preferential catabolism; the latter point has often been overlooked when examining isozyme repertoires. In addition, a thorough analysis is made of the sub-cellular localisation of isozymes and the genetic and molecular basis for the specificity of the positioning of the isozymes within the cell—a topic of profound importance when considering the mechanisms of enzyme regulation. In this last section the authors indicate those areas of isozymology which will probably be the major research directions of the future. This clearly written and well organised overview of many facets of isozymes and haemoglobins should be of interest to biologists and biochemists representing a wide spectrum of research interests.

Gregory S. Whitt

An Introduction to Medical Physics. By Edwin G. A. Aird. Pp. viii+293. (Heinemann Medical: London, May 1975.) £4.95.

THIS book deals in a straight-forward way with those applications of physics in medicine that are most used. It is broadly divided into "Ionising Radiations and their Uses" and "Other Applications of Physical", roughly 2 : 1 giving a rather classical, 'measure the dose', hospital physics flavour. It reads easily, however, and in the radiation sections, the clarity and succinct presentation were appreciated. In the second part some of the information could have been made more up-to-date—although this is where the subject is expanding very rapidly, for example, chapter 8 on the use of ultrasonics.

The discussion of physiological measurement is most welcome for it is here that physics and physiology merge. The emphasis, however, is entirely on the technical—as it is throughout the book—with no attempt to show, even in a small way, the conceptual stimulus of physics.

The number of errors detected were small, an indication of the care in both writing and production, although the confusion between longitudinal and transverse orbits of the medium in the propagation of sound waves is too fundamental to go unremarked (p179) and p184) and should be corrected as soon as possible.

Overall, the book demonstrates clearly one facet of the applications of physics in the community. It can be warmly recommended to its author's intended audience—hospital technicians, radiographers and graduate or specialist nurses. For a graduate scientist entering the field it is well worth reading quickly as the introduction it purports to be.

R. G. Gosling

Comprehensive Chemical Kinetics. Volume 14: Degradation of Polymers. By C. H. Bamford and C. F. H. Tipper. Pp. xv+562. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl.200; \$83.50.

THIS series has been a brave and generally very successful attempt to review the whole field, which is loosely described as chemical kinetics. The present volume describes the very complex reactions which cause degradation in synthetic polymers and it is not surprising that the emphasis is on mechanisms rather than detailed kinetics. Indeed, few polymer degradation reactions are well enough understood to enable precise descrip-

tion of the kinetics of the elementary steps. The book has four chapters, classified according to the mode of initiation of the degradation reaction. The first, dealing with thermal degradations contains an excellent introduction to the kinetic models used in describing these reactions, followed by detailed discussions of the behaviour of a wide range of common polymer types. A similar pattern is followed in chapters two and three, which describe degradations induced by high energy radiation and by photochemical methods. The final chapter is concerned with the complex reactions which can occur when polymer degradation occurs in the presence of oxygen or ozone.

Books brief

As a whole this book is clearly and concisely written and all the authors have done a good job in reviewing their fields. There is much material of interest both to the expert and the beginner. One serious criticism is that the literature coverage does not extend beyond 1971. A publication delay of four years is excessive by any standards and together with the price makes the value of this book rather doubtful, even for specialist libraries.

N. C. Billingham

Films on Solid Surfaces: The Physics and Chemistry of Physical Adsorption. By J. G. Dash. Pp. xi+273. (Academic: New York and London, September 1975.) \$26; £13.

THE powerful battery of techniques both experimental and theoretical, which have evolved over the past 20 years has enabled the field of surface studies to progress from a state consisting largely of guesswork to one of increasingly detailed understanding. Attempts on the experimental side in the early 1950s to verify the predictions of 'two-dimensional theory' were generally unsuccessful and the apparently confident prediction—that perfect two-dimensional states, for example, crystals, magnetic arrays, superconductors, could not exist—could hardly be questioned. Thanks to the impressive advances in methods such as electron spectroscopy, field-emission and field-ion microscopy, molecular scattering and to the ease with which ultra-high vacuum conditions can be established,

an increasing amount of reliable information on adsorbed films is accumulating. There is an increasing interest in the study of the statistical thermodynamics of physisorption and on the thermodynamics of non-interacting monolayers, which latter relates to the important question of mobility of adsorbed atoms in the formation of surface films. Much of the progress on the experimental side has come from physicists for whom a volume such as the present one will form an invaluable introduction to some of the more chemical aspects of the subject. The introduction to theoretical studies is clearly written and a useful selection of experimental results—limited presumably to keep the overall length in check—gives an excellent view of the present state of the field.

O.S. Heavens

The Heavy Transition Elements. (A Macmillan Chemistry Text.) By S. A. Cotton and F. A. Hart. Pp. x+272. (Macmillan: London and Basingstoke, September 1975.) Hard cover £8.95; paper cover £4.95.

THIS book is a logical sequel to a cognate volume from the same publishers, which dealt with the 3d transition elements. A first impression is that of the wealth of descriptive inorganic chemistry contained within its covers. Here is a truly up-to-date account of the significant chemistry of these elements, without the loss of older well-established facts.

The choice of structural figures to illustrate chemical points is excellent; and chapters, sections and subsections of the text are well organised and, helpfully, are consistent from chapter to chapter wherever possible. At the start of each chapter there is a table of coordination number against oxidation state for the corresponding 3d element of the group, which gives a useful summary for comparison purposes.

The book, in addition to covering the 4d and 5d elements from zirconium and hafnium through to silver and gold, has excellent chapters on the lanthanides and actinides, and another chapter devoted to metal complexes containing π -bonding ligands.

Although not intended as a work of reference, the bibliography is adequate, and each chapter is referenced with a range of appropriate review articles.

This book is a must for libraries, and very desirable for the bookshelves of individual inorganic chemists.

Edward Abel

obituary

Detlev Wulf Bronk died at New York Hospital on November 17, 1975 from complications following a stroke. He was 78 years old. Bronk was one of the outstanding leaders of science in the United States, and his influence also reached out far into the international scene. In the course of his career, he was active and well-known in scientific organisations in Europe, Asia, and Latin America as well as North America.

His initial scientific interest was in electrical engineering and physics, but he soon became committed to biological problems. As a National Research Council (NRC) fellow in the late 1920s, he worked with A. V. Hill at the University of London on studies of the production of heat in muscles and with Edgar Douglas Adrian, now Baron Adrian of Cambridge, on the development of techniques of recording the electrical activity of single nerve fibres.

Because of these interests, he was appointed director of the newly established Eldridge Reeves Johnson Foundation for Medical Physics at the University of Pennsylvania in 1929. This remained his base until he left to become president of The Johns Hopkins University in 1949.

During World War II, he became involved in aviation medicine based on a combination of prior interests, which included a period as a naval air cadet in 1917-18. This activity not only carried him to a number of theatres of combat

but involved him in a variety of applied scientific enterprises which made it natural for him to assume the chairmanship of the National Research Council of the National Academy of Sciences (NAS) at the end of the war. At that time the Council was the major working arm of the Academy.

In 1950, he was elected president of the NAS, where he spent a substantial fraction of his time participating as a leader in the very rapid development of science in the United States that accompanied the great growth of government investment in science following World War II. In this connection he served as a member of the National Science Board of the National Science Foundation for fourteen years and was its chairman between 1956 and 1964.

In 1953, he was asked to become head of the Rockefeller Institute for Medical Research, on whose board he had served since 1946. He accepted this post with the understanding that he would be in a position to develop a graduate program in parallel to the postdoctorate educational program which the Institute had supported since its creation early in the century. The Institute eventually changed its name to The Rockefeller University—the first purely graduate university in the United States. He retired as president of Rockefeller University in 1968, but remained exceedingly active in national and international scientific affairs up to the time of his death. In fact, his illness, which came

upon him quite suddenly, took place just a few weeks after his return from the Soviet Union, where he had participated, as a foreign member, in the 250th anniversary celebration of the Soviet Academy of Sciences.

Detlev Bronk was one of the most honoured scientists of our time, having participated and received almost all of the awards in almost all of the activities that lay within his sphere of professional concern. Those who knew him well and worked with him, however, admired him most for his intense interest in individuals as such. He had an enormous circle of close friends and the gift of bringing them together into effective working partnership on countless occasions. This ability served the interests of the NAS exceedingly well, since he employed it, in a most remarkable way, to make that organisation a highly effective advisor both at home and abroad.

Closely related to his interest in people was a profound conviction that the pursuit of science has its own intrinsic rewards which are reflected in its influence on human enlightenment. He understood quite clearly that, while science could serve as handmaiden to the applied arts, it must not become a slave to them. He believed deeply that the freedom of science is one of the essential freedoms which must be sustained if mankind is to become a more perfect reflection of the Creator.

Frederick Seitz

announcements

Appointment

Professor R. N. Hazeldine, F.R.S., Head of the Department of Chemistry at the University of Manchester Institute of Science and Technology, has been appointed to succeed **Lord Bowden** as Principal of UMIST in September 1976.

Reports and publications

Other countries

United States Department of the Interior: Geological Survey. Professional Paper 914: Geologic Considerations for Redevelopment Planning of Managua, Nicaragua, following the 1972 Earthquake. By Henry R. Schmoll, Richard D. Krushensky and Ernest Dobrovolsky. Pp. iii + 23. Professional Paper 907-A, B. Grade and Tonnage Relationships Among Copper Deposits. By D. A. Singer, Dennis P. Cox and Lawrence J. Drew and Geochemical Exploration Techniques Applicable in the Search for Copper Deposits. By Maurice A. Chaffee. (Geology and Resources of Copper Deposits) Pp. vii + A11, iii + B26. (Washington, DC: US Government Printing Office, 1975.) [512]

Person to Person

Scientist going on sabbatical leave to Stanford would like to exchange houses with someone in the Stanford area. House near St Albans, within easy reach of London, has 3 bedrooms, central heating, garage and schools nearby. Available about mid-August 1976 for 12 months. (Dr G. R. Banks, National Institute for Medical Research, Mill Hill, London NW7 1AA.)

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

United States Department of the Interior: Geological Survey. Water-Supply Paper 1827-B: Reactions of Aqueous Aluminum Species at Mineral Surfaces. By D. W. Brown and J. D. Hem. Pp. iv + 48. Water-Supply Paper 2109: Surface Water Supply of the United States, 1966-70, Part 3: Ohio River Basin. Vol. 3: Ohio River Basin from Louisville, Kentucky, to Wabash River. Pp. viii + 633. Water-Supply Paper 2150: Quality of Surface Waters of the United States, 1969. Parts 12-16: North Pacific Slope Basins, Alaska, Hawaii, and Other Pacific Areas. Pp. xiii + 480. \$3.50. Professional Paper 712-C: Hydrogeologic and Hydrochemical Framework, South-Central Great Basin, Nevada-California, with Special Reference to the Nevada Test Site. By Isaac J. Winograd and William Thordarson. Pp. vii + 126 + 3 plates. Professional Paper 756: Middle Jurassic (Bajocian) Ammonites from Eastern Oregon. By Ralph W. Imlay. Pp. iv + 100 + 47 plates. \$2.50. Professional Paper 843-A: Distribution of Gold and Other Ore-Related Bodies in the Oxidized Zone at Goldfield, Nevada. By R. P. Ashley and J. P. Albers. Pp. iii + 48 + 4 plates. Professional Paper 878: Evaluation of Ground-Water Degradation Resulting from Waste Disposal to Alluvium near Barstow, California. By Jerry L. Hughes. Pp. iv + 33. Professional Paper 888: Tectonic Studies of the Berkshire Massif, Western Massachusetts, Connecticut, and Vermont. Pp. iii + 106. (Washington, DC: US Government Printing Office, 1974 and 1975.) [812]

Bracteatum: a Potential Domestic Source of Codeine. Pp. 16. (St. Louis, Missouri: Mallinckrodt, Inc., 1975.) [812]